Article

Morpho-physiological changes of *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst. seedlings to different nursery light environments

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Abstract:

In order to determine if the management of nursery light conditions has potential to manipulate morpho-physiological characteristics of seedling quality, in this study the morpho-physiological changes of *Prosopis laevigata* seedlings subjected to varied light environments, both under full sun and shading with nets of different colors (blue, black, red, and green) during nursery production, are measured. We evaluated their growth, in terms of biomass gain per seedling; their morphology, trough biomass allocation patterns, and by calculating the specific leaf area, and their photosynthetic efficiency as net assimilation rates. Seedling grown under red net attained the highest growth, 24 % higher compared to seedlings grown under full sunlight. Biomass allocation patterns were affected. Under full sun, the seedlings allocated less than 50 % of the total biomass to shoot and increased proportionally the root biomass, whereas a shading with nets, regardless of their color, inverted the patterns, promoting a higher biomass allocation to the shoot (more than 60 %). Under full sunlight, specific leaf area diminished, but under shading it increased up to 106 % under the black net. The higher net assimilation rates were obtained by seedlings under full sunlight; however, better photosynthetic efficiency was achieved by seedlings grown under the red net due to their higher growth. It is concluded that it is possible to manipulate the morpho-physiological characteristics of *P. laevigata* seedling quality by managing the nursery light conditions.

Key words: Acclimation, tree seedling quality, mesquite, phenotypic plasticity, reforestation, forest nurseries.

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Introduction

From the high fragmentation and reduction of forest populations in the arid and semi-arid zones of north-central Mexico, mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M. C. Johnst.)) is one of the main arboreal or shrub species that are most commonly used for forest ecosystem restauration (Ríos *et al*., 2012).

However, reforestation with this species has not been economically or ecologically profitable because of the high mortality rates and poor growth of the plants in the field. This performance is associated with a synergistic effect between adverse environmental conditions in the field and the use of a nursery plant with a low capacity for acclimatization to the heterogeneity of edaphoclimatic conditions in the sites to be planted (Ríos *et al.,* 2012; Contreras, 2017).

To improve the performance of reforestation with mesquite, the need to improve the quality of the plant produced in the nursery, in terms of morpho-physiological attributes, has been emphasized, through the study of the effectiveness of some cultural practices; the main interest has been concentrated in its effect to promote high rates of growth of both the air and the radical component and the balance between them. The purpose is to obtain sizes according to the standard NMX-AA-170-SCFI-2016 (DOF, 2016) of 20-30 cm in height of the aerial part and diameter in the neck of the root of 3.5 mm or more, inside of a production cycle of four to five months.

Nursery work with the species referred to so far (Prieto *et al*., 2013) has not had the appropriate approach to define one or more practices with which the growth and development of the plants are modified, according to quality specifications in the morpho-physiological attributes defined by the conditions of the site to be reforested; that is, if a large plant is required, with a robust stem, with a high foliar area for sites with plant or grazing competition, or a small plant, with a robust stem and a radical system well enough for dry sites. This is due to the fact that, both in field and laboratory conditions, the growth and development of the plants are affected by various environmental factors, of which one is the available solar light.

Photoperiod, quality and quantity of sunlight directly affect the photosynthesis of plants and other phenotypic and functional characteristics (Peixoto *et al.,* 2014); therefore, the effectiveness of a cultural practice could vary according to the light requirements of each species in the nursery, especially in the aspects of quality and quantity of solar radiation. For example, in the production of seedlings of *Nothofagus leonii* Espinosa with different levels of shade and fertilization there were morphological and physiological changes only due to shade effect (Santelices *et al.,* 2015); instead, seedlings of *Shorea leprosula* Miq. they increased their biomass by the addition of phosphorus when they grew in high luminosity environments (Brearley *et al.,* 2007).

The phenotypic responses of plants to varied light conditions have been used to modify their morpho-physiological characteristics according to desired quality specifications; In this regard, Kelly *et al*. (2015) analyzed the impact of quantity and quality of light to promote in *Populus tremuloides* Michx. seedlings. a high ratio between the proportion of root and aerial, and an increase in the levels of non-structural carbohydrates of the roots, since these features predict a satisfactory performance in the field in soil reclamation works.

This approach could be implemented during the production of mesquite plants in nursery to manipulate their growth and induce morpho-physiological characteristics more appropriate to the conditions of the possible transplant site, mainly given the availability of low-cost inputs in the market that modify the available light; one of them is shade meshes, which have been widely used in horticulture to improve the quality of crops and increase their yield through color and their associated photoselectivity (Stamps, 2009); however, your assessment is necessary. For the above reason, the study described below had the objective of morphologically and physiologically quantifying the changes of mesquite seedlings subjected to different conditions of sunlight.

Materials and Methods

Propagation of mesquite seedlings

At the beginning of March 2016, about 300 mesquite seedlings (*Prosopis laevigata*) were grown from seed in the forest nursery of the *Campo Experimental Valle del Guadiana* (*Valle del Guadiana Experimental* station) of the *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias* (National Institute for Forestry, Agriculture and Livestock Research) (INIFAP), in *Durango*, Mexico (23°59'17.1" N, 104° 37'35.7" W, 1 880 masl).

The mesquite seeds, whose germination was verified around 95 %, came from natural stands distributed in the municipality of *Aldama*, *Chihuahua*, Mexico. Seeding was direct in black rigid plastic tubes of 380 mL filled with a substrate composed of peat, vermiculite and perlite in 2: 1: 1 proportions. Prior to sowing, according to the recommendations of Prieto *et al.* (2012), the seeds received a pregerminative treatment with hot water and were treated with Benomyl: Methyl 1-(butylcarbomoyl) benzimidazol-2-il carbamate 50 % in a dose of 2 g L^{-1}. During the germination period constant irrigations were applied to saturation.

Treatments and experimental design

Four weeks later, once the germination and emergence were stabilized, 225 seedlings uniform in height (~ 5 cm) were randomly selected and organized into groups of 15, which corresponded to the size of the experimental unit.

Each of the five environmental conditions of light (open air or open sky, blue shadow mesh, black shadow mesh, red shadow mesh and green shadow mesh) had three repeats and were assigned to the experimental units by a completely random design. The meshes used were of the brand PROTEXOL® 80 %.

Metallic structures in the form of a rectangular prism were covered completely by the respective mesh, to which their transmittance (T; %) was determined; for this, the photosynthetically active radiation (RFA) was measured inside the structure and outside it with a ceptometer (Model SF-80, Decagon Pullman) and the formula T = internal RAF / external RAF was used. The meshes presented the following transmittance values: blue mesh, 26 %; black, 24 %; red, 40 %; and green, 25 %.

Under all conditions the temperature value (°C), relative humidity (%) and global radiation (Wm^{-2}) were monitored daily. The first two variables were measured with a digital hygrothermometer (Model 445702, Extech Instruments) and the last with a pyranometer (Model SP Lite, Kipp & Zonen, Delf). The global radiation values were multiplied by a constant of 0.50 to determine the values of RFA, which, in turn, multiplied by 4.15 to transform them to $\mu mol\ m^{-2}s^{-1}$ (Terradas, 2001). After that, the RFA value of the meshes was calculated according to the respective percentage of transmittance. Table 1 shows the values of the monitored climatic variables.

Table 1. Values of the climatic variables monitored in each of the environmental light conditions.

Climatic variable	Environmental light conditions				
	Weather	Blue shadow mesh	Black shadow mesh	Red shadow mesh	Green shadow mesh
[1] Maximum T (°C)	28.1	41.2	37.3	40.4	37.3
Minimum T (°C)	14.2	10.5	10.9	10.3	10.3
[2] Maximum HR (%)	67.8	95.8	95.2	94.8	95.2
Minimum HR (%)	15.0	18.8	22.5	18.78	22.5
[3] Average RFA (μmol $m^{-2}s^{-1}$)	1485.6	386.2	356.5	594.2	371.4

[1]T = Temperature; [2]HR = Relative humidity; [3]RFA = Photosynthetically active radiation.

During the growth period in each light condition (three months), the seedlings were watered twice a week at saturation and were fertilized once a week with a general purpose water-soluble fertilizer 20-10-20 [20 N: 10 P_2O_5: 20 K_2O + microelements] (Peters Professional®), at a nitrogen base concentration of 150 mg L^{-1}.

Evaluation of growth, morphology and photosynthetic efficiency

Seedling growth was analyzed in terms of biomass gain at plant level and measured as relative growth rate (TCR, in mg g^{-1} day^{-1}). The morphology was determined by calculating the specific leaf area (AFE, cm^{-2} g^{-1}) and through biomass allocation patterns at the level of stem and root leaves, so the proportion of leaf biomass (PBF; %), the proportion of stem biomass (PBT; %) and the proportion of radical biomass (PBR; %). The photosynthetic efficiency was evaluated with the recording of the net assimilation rate (TAN, mg cm^{-2} day^{-1}). The calculation of the response variables required the destructive sampling of a sample of 15 seedlings of each light condition, at the beginning (March, 2016) and at the end of the trial (June 2016). The procedure began with the measurement of leaf area using a foliar area meter (CI-202, CID, Inc.); then, continued with the separation of the other components of the seedlings (stem and root), and then finished with the drying of these (including the leaves) in a forced air stove (Felisa FE133A) at 70 °C for 72 h, and its weighing in an analytical balance (*Ohaus Pioneer* PA224C).

The observations of foliar area and weight of dry biomass obtained were used to estimate the values of each variable according to the formulas described by Hunt *et al.* (2002) presented below:

$$TCR = \frac{ln\, PST_2 - lnPST_1}{\Delta t\ (days)}$$

Where:

PST_1 and PST_2 = Total dry weight of the seedlings in the initial and final assessment

Δt = Time elapsed between both assessments (90 days)

$$PBF\ (\%) = \frac{Leaf\ dry\ weight}{Total\ dry\ weight} * 100$$

$$PBT\ (\%) = \frac{Stem\ dry\ weight}{Total\ dry\ weight} * 100$$

$$PBR\ (\%) = \frac{Root\ dry\ weight}{Total\ dry\ weight} * 100$$

$$AFE = \frac{Foliar\ area\ (cm^2)}{Foliar\ dry\ weight\ (g)}$$

$$TAN = \frac{(PST_2 - PST_1)\ (ln\ AF_2 - ln\ AF_1)}{(AF_2 - AF_1)(T_2 - T_1)}$$

Where:

PST = Total dry weight of seedlings in absolute and logarithmic (*ln*) values

AF = Foliar area of seedlings in absolute and logarithmic (*ln*) values

T = Time. In all cases 1 and 2 refer to the initial and final assessment with a 90-day period

Statistical analysis

A non-parametric analysis of variance was made through the Kruskal-Wallis test. When necessary, means were compared by pairs. *P* <0.05 statistical differences were taken as significant. Data are presented as means ± standard deviation. The statistical analysis was made in infoStat (Di Rienzo *et al.,* 2012).

Results

The growth, morphology and photosynthetic efficiency of the mesquite seedlings was modified according to the light condition in which they grew, therefore, all the evaluated variables (TCR, PBF, PBT, PBR, AFE and TAN) registered highly significant differences ($P \geq 0.0001$).

The highest relative growth rate was in the seedlings that grew in the red shadow mesh. The value obtained in this condition of 7.23 mg g^{-1} day^{-1} was 24 % higher than the value obtained in the weather condition, which recorded the lowest rate of 5.85 mg g^{-1} day^{-1} (Table 2).

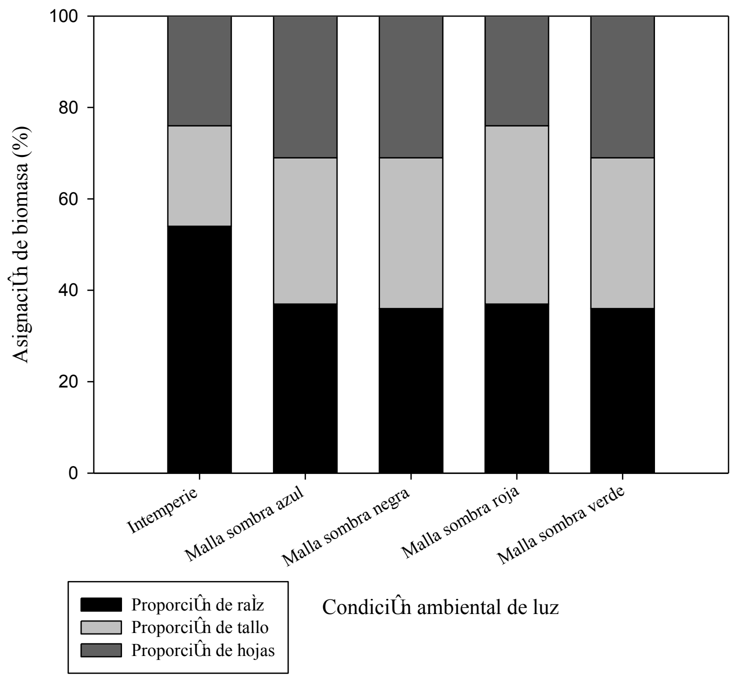
Table 2. Average values of the growth variables, biomass allocation and photosynthetic efficiency assessed in mesquite (*Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M. C. Johnst.) seedlings subjected to varied light conditions.

Response variable	**Environmental light conditions**				
	Weather	**Blue shadow mesh**	**Black shadow mesh**	**Red shadow mesh**	**Green shadow mesh**
TCR[1] (mg g^{-1} day^{-1})	5.85±0.22a	6.90±0.22b	6.60±0.30b	7.23±0.33c	6.90±0.30b
AFE[2] (cm^{-2} g^{-1})	64.18±18.52a	108.16±12.52b	132.50±15.96c	86.27±12.44a	121.35±16.24b
TAN[3] (mg cm^{-2} day^{-1})	1.55±0.93a	0.89±0.21b	0.68±0.16b	1.32±0.27a	0.81±0.15b

Means with the same letters between lines are not significantly different (P>0.05).

[1]TCR = relative growth rate; [2]AFE = specific foliar area; [3]TAN = net assimilation rate.

In regard to biomass allocation, more than 30 % of that produced by seedlings belonged to foliar biomass in most of the shadow mesh conditions, except for the red one, which recorded a very similar value to the weather condition with 24 % of PBF. Such response was the opposite in PBT, since it accumulated 39 % of biomass in the stem, which surpassed the values of the rest of the conditions, mainly of the weather ones in which the seedlings produced there allocated less than 25 % of the total biomass to such tissue. Nevertheless, PBR showed a different effect in the latter from what was previously in record. In this case, seedlings allocated more than half of their biomass to the root, thus overcoming with more than 50% the numbers from the other light conditions (Figure 1).



Asignación de biomasa = Biomass allocation; Condición ambiental de luz = Environmental light conditions; Intemperie = Weather; Malla sombra azul = Blue shadow mesh; Malla sombra negra = Black shadow mesh; Malla sombra roja = Red shadow mesh; Malla sombra verde = Green shadow mesh; Proporción de raíz = Root proportion; Proporción de tallo = Stem proportion; Proporción de hojas = Leaves proportion.

Figure 1. Biomass allocation patterns in *Prosopis laevigate* (Humb. & Bonpl. ex Willd.) MC. Johnst. seedlings in contrasting light conditions at the nursery.

On the other hand, the AFE variable tended to decrease as the level of radiation increased, so it had superiority in the seedlings that grew in black shadow mesh, with recorded values of 132.50 cm^{-2} g^{-1}, which were 106 % more high compared to the seedlings of the weather, which had the lowest values of 64.18 cm^{-2} g^{-1} (Table 2).

Finally, physiologically mesquite seedlings underwent changes in their photosynthetic efficiency depending on the light environment to which they were exposed. When the seedlings grew outdoors, they maintained high net assimilation rates of 1.55 mg cm^{-2} day^{-1}, but they were reduced to 128 % in the black shade mesh environment that registered the minimum value of 0.68 mg cm^{-2} day^{-1}(Table 2).

Discussion

While the mesquite seedlings remained in the nursery, the environmental conditions of light modified their growth, morphology and photosynthetic efficiency. One of the factors involved in this effect was the microenvironment generated in each condition, specifically as regards temperature and humidity. As it is widely described in horticulture (Stamps, 2009), the micro-environmental conditions of temperature and humidity caused by the shaded mesh (Table 1) apparently favored the growth of the specimens studied, those of the red mesh in particular, whose growth was 24 % higher compared to those that grew outdoors.

However, the evidence obtained suggests a greater attribution of the growth and the morphological and physiological modifications of the seedlings to the amount and quality of solar irradiation that they received in each condition, in particular in the shadow mesh in which different levels of radiation and possibly different photoselectivity depending on the color of the mesh.

This point is consistent with the aspects that are attributed to the use of shade shades of colors in the cultivation of vegetables (Stamps, 2009), with experimentally demonstrated in nursery conditions with *Bauhinia variegata* (L.) Benth seedlings. (Mazzini and Pivetta, 2014) and with the responses of mesquite seedlings in the variables evaluated. In this regard, the shade meshes promoted that morphologically the seedlings would manifest the typical phenotypic responses of acclimatization of a pioneer species, before a heterogeneous light environment (Pearcy, 2007). Low levels of radiation resulted in a greater allocation of biomass to the leaves and stems, while, at high levels, a greater allocation of biomass to the root was favored.

In this way, when the seedlings grew in the meshes, more than 60 % of the biomass was assigned to the aerial part (including leaves and stem), while, in the open, more than 50 % of the biomass was destined to the root (Figure 1).

These changes in the root / aerial part relationship can be attributed, although this aspect was not evaluated, to variations in the amount of light received in the red / far red relationship, since this factor is important for phytochromatically mediated responses (Batschauer, 1998).

Around this, Kelly *et al*. (2015) sustained with *Populus tremuloides* seedlings, that a high red / red distant ratio, favors the growth and development of the root; on the other hand, a low red / red ratio low, stimulates growth and aerial development. Thus, the weather condition supposes a high exposure to this relation, while the shadow mesh conditions suppose a low exposure, which in turn could vary towards lower levels due to the color of the meshes, as it is demonstrated in a study conducted by Shahak *et al.* (2004).

However, contrary to what was previously stated, Kelly *et al.* (2009) demonstrated experimentally, opposite changes between the biomass allocation patterns of six subtropical Australian species subject to different light gradients, and those promoted in mesquite seedlings, since in these species a greater availability of light results in an increase of aerial biomass and a decrease of the biomass assigned to the root.

This contrariety of responses can be associated with the degree of phenotypic plasticity of the species, since Cheng *et al.* (2013) in a study whose context is similar to that of Kelly *et al.* (2009) and that of this work, reported a low phenotypic plasticity of the patterns of allocation of biomass to light in five subtropical evergreen broadleaf species.

On the other hand, at leaf level, the variations in the availability of light induced in each evaluated condition led to morphological changes, in such a way that a scarce availability of light made the leaves thinner and increased their specific leaf area (AFE), while a greater supply of light resulted in a decrease in AFE, and, therefore, in a thickening of the leaves. The aforementioned is verified with the results of the black shadow mesh as well as with the weathering, since each one represented each case respectively (Table 2).

Several authors agree with the findings of this work and argue that this effect on the leaves corresponds to morpho-functional changes that the plants promote, to use more efficiently the available radiation and thus maintain an adequate photosynthetic capacity, depending on the availability of it, either by increasing the leaf surface by quantity of inverted biomass or by increasing the density of the leaves (Cheng *et al.,* 2013; Yang *et al*., 2013; Tang *et al.,* 2015).

Physiologically, the results obtained show that mesquite seedlings underwent changes in their photosynthetic efficiency depending on the light environment to which they were exposed. The fact that the TAN, when grown outdoors, was 128 % higher than the black shade mesh, reflects that a greater availability of light promotes high carbon gains at the plant level. From there, it would be expected that the more the passage of light with the meshes is limited, the lower the carbon gains. Although the TAN values of the weathering condition suggest a greater carbon gain in the plants because they are the highest (although they were not statistically different from those of the red mesh), their growth results compared to that of the seedlings of The red mesh contradicts this assumption, since in fact the meshes of the meshes were the fastest growing, recording a 24 % higher growth (Table 2).

This positive effect of the red mesh could be attributed to the promotion of a more adequate balance between the carbon gains, due to the TAN and the carbon losses, associated with respiration, which were probably lower in comparison with the weathering seedlings; this is because in the shade the low availability of light limits the carbon gain, but it reduces the respiration rates, so that the plants maintain a positive carbon balance (Pearcy, 2007). In addition, as reported in a study with seedlings of *Torreya grandis* Fortune ex Lindl. in which the optimal intensity of light was determined (Tang *et al.,* 2015), the shade generated by the mesh could have prevented damage by photoinhibition and increased the efficiency of the leaves to capture and absorb more radiation with the increase in value of AFE, which, although it was lower compared to the seedlings of the other shade meshes, surpassed that of the weather in 34 %, although they were statistically similar (Table 2).

Therefore, the values of TAN and AFE could have contributed to the growth of the red mesh seedlings, independently of the degree of variation that each one has in explaining the growth, of which it is an aspect in which there is controversy given the nature of each variable and the work done in this regard, for example Villar *et al.* (2005) and Tomlinson *et al.* (2014).

Finally, it should be noted that the seedlings of all the conditions evaluated met the minimum morphological quality standards required by the NMX-AA-170-SCFI-2016 Mexican Standard (DOF, 2016) within a production cycle of four months. Hence, the findings of this study have practical implications for reforestation work, since characteristics of nursery seedlings can be manipulated according to morphological and physiological specifications defined by the edaphoclimatic conditions of the site to be reforested.

However, new experimental approaches may be required to evaluate the performance of plants in the field in a variety of environmental conditions, after being acclimated to a specific area of light in the nursery.

Conclusions

Mesquite seedlings have a good ability for acclimatization to contrasting light conditions (which is an expression of their phenotypic plasticity) during their growth period in the nursery. A high exposure to light reduces the growth and the formation of aerial biomass, but stimulates a greater formation of radical biomass. On the other hand, shade shading, regardless of its color, promotes greater growth and inverts the biomass allocation patterns, favoring the formation of biomass in the aerial component; it undergoes morphological changes at the foliar level with the increase of its foliar surface, which is contrary to the thickening that the leaves suffer when they are exposed to high levels of radiation without the protection of mesh, of which, the red one increases the photosynthetic efficiency of the plants, thus allowing them greater carbon gains.

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Conflict of interests

The authors declare no conflict of interests.

Contribution by author

Erickson Basave Villalobos: planning of the experiment and writing of the manuscript; Sergio Rosales Mata: data processing and statistical analysis; José Á Sigala Rodríguez: data statistical analysis and review of the manuscript; Celi G. Calixto Valencia: conduction and technical supervision of the experiment; Homero Sarmiento López: assessment of the experiment and review of the manuscript.