AGRO PRODUCTIVIDAD

Phenotypic plasticity and biomass allocation in fertilized *Quercus variabilis* Blume seedlings

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Citation: Flores, A., Corona-Ambriz, A., Méndez-González, J., & Muñoz-Flores, H. J. (2024). Phenotypic plasticity and biomass allocation in fertilized *Quercus variabilis* Blume seedlings. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i2.2674

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: September 06, 2023.
Accepted: January 15, 2024.
Published on-line: March XX, 2024.

Agro Productividad, 17(2). February. 2024. pp: 71-89.

ABSTRACT

Objective: To determine the phenotypic plasticity between fertilized (F) and unfertilized (UF) *Q. variabilis* seedlings developed in a nursery using small, medium, and large seeds.

Design/methodology/approach: By analyzing the phenotypic traits of growth (height and diameter at root collar) and root and stem dry biomass, plasticity indices and allocation patterns were assessed, and phenotypic traits in which the interaction effect of the seedling fertilization and seed size was observed.

Results: The results showed significant differences (p-value≤0.05) in the growth and biomass traits except for the root dry biomass, and significant differences were found both in the fertilization and seed size factors and in the fertilization and seed size interaction in phenotypic traits. For plasticity, all traits showed marked changes in response to the nutrient application, and small seeds had the highest plasticity indices. With respect to phenotypic change indices, medium seeds reached the highest values (0.94 for height and 0.92 for diameter). Large seeds recorded the highest index for root dry biomass (1.01), and medium seeds had the highest values of plasticity for stem dry biomass (0.81). Allometric differences were observed (intercepts were 1.8374 and 3.4956, and slopes were 4.0943 and 1.7038 for UF and F seedlings, respectively), and variations in the study factors (fertilization and seed size).

Limitations on study/implications: In order to improve the survival of plants in the field it is necessary to use seeds with high quality.

Findings/conclusions: Fertilization of *Q. variabilis* seedlings increases their biomass production, which allows for greater growth and survival compared to unfertilized ones.

Keywords: Environmental variation, Forest conservation, Phenotypic variation, Seed size.

INTRODUCTION

Quercus genus is important in the temperate and tropical forests of the northern hemisphere. It comprises approximately 450 species, with differences in their flowering, fruiting, and maturation, due to their phylogenetics and ecological factors present in their distribution (Vinha *et al.*, 2016). In China, there are 40 oak species, which stand out for their role in maintaining biodiversity and the stability of the ecosystems they form (Sun *et al.*, 2020). In this country, the study of its taxa is a current issue for conservation in some of oak species, *e.g.*, *Quercus variabilis* Blume, *Q. mongolica* Fisch. ex Ledeb., *Q. acutissima* Carruth.

Quercus variabilis has high ecological, economic, and cultural value in China (Gao *et al.*, 2018). To conserve this resource in the face of the constant degradation of the forests it harbors, some research has been carried out, *i.e.*, the current status and potential distribution under climate change scenarios (Sun *et al.*, 2020), factors affecting seedling regeneration in different climatic regions (Wu *et al.*, 2013), inter-and intraspecific phenotypic variation (Feng *et al.*, 2021), and population structure and dynamics (Zhang *et al.*, 2008). These topics were part of the global strategy for plant conservation in China (2011-2020), but further research is still required to maintain and rehabilitate the current population of the species.

In plants, nutrients not only play an important role in tissue development but also help them to adapt to environmental variations. This has highlighted the importance of fertilization for nutrient supply given the continuing effects of climate change. For example, global warming can reduce nutrient reabsorption, while drought can accelerate leaf senescence and increase reabsorption (Rivero *et al.*, 2007). Thermal changes brought about by global warming affect the distribution and abundance of plants, in addition to their respiration, and limit the availability of minerals in the soil that can reduce their growth responses and biomass production. Increased atmospheric humidity leads to change in nutrient storage, growth, and development, which can be reversed by supplying macronutrients (Oksanen *et al.*, 2018). Studying the adaptive potential of forest species and their response to stimuli such as fertilization is a priority for their continuity and to achieve greater survival and growth at their planting sites.

Currently, the drought tolerance evaluation of forest species to climate change scenario is increasingly relevant. In this regard, using available data from online repositories to analyze species responses is a valid method (Mendeley, Zenodo), given that these are databases considered to have the potential to assess their plastic responses in different climatic gradients (Vizcaíno-Palomar *et al.*, 2019).

Studying a species' phenotypic plasticity (the capacity to alter phenotype in direct response to environmental changes (Costa, 2021)) allows to understand and predict its response to changes in the environmental factors in which it is found, and the performance of the environmental and genetic components (Pazzaglia *et al.*, 2021). Seed size is an important factor for analyzing phenotypic plasticity since it is a variable that evolves in responding to different environmental stresses (Li *et al.*, 2021). There is limited research available for *Q. variabilis* on plasticity in the last decade, based on different seed sizes (Xu *et al.*, 2015), which makes it difficult to understand how the species will adapt to different scenarios.

Therefore, the objective of this research was to determine the phenotypic plasticity of fertilized and unfertilized *Q. variabilis* seedlings produced in a nursery from small, medium, and large seeds.

MATERIALS Y METHODS

Plant material data

Data collected on fertilized and unfertilized seedlings were obtained from the repository (greenhouse experiment) and used in this study for analyze the *Q. variabilis* plasticity. At present, databases from online repositories is considered to have the potential to assess plastic responses of plants. For consultation, this information is available at the Mendeley repository (https://doi.org/10.17632/krgxdd2rtp.2). From this database, four variables were selected: two phenotypic growth traits (height (cm), and root collar diameter (cm)) and two biomass traits (root dry biomass (g), and stem dry biomass (g)). These variables were chosen because they have been used to evaluate the plasticity, and their variations under different environments.

The greenhouse experiment was undertaken during 2014 to test the impact of fertilization in seedling morphology and nutrition in the nursery and outplanting performance (Shi *et al.*, 2019). The trial was established in the Beijing Forestry University near Jiufeng Mountain, Beijing having 28.5 °C day and 16.5 °C night temperature and 84.7% relative humidity. Seedlings were produced from three seeds of different sizes: small (2.88±0.09 g), medium (4.18±0.10 g), and large (5.52±0.27 g), which were defined using fresh weight (g) and by performing cluster analysis. Four hundred seeds ofeach size class were sown in cylindrical, hard plastic D60 containers: one seed per container (1-2 cm of depth). The container diameter was 6.4 cm and the depth was 36.0 cm, resulting in a volume of 983 ml. The growing medium was a 3:1 (v:v) mixture of peat (pH 6.0, screening 0-6mm) and perlite (5 mm diameter).Seventy-five seedlings (25 individuals of each seed size) were fertilized with 20 ml 20N-20P-20K solution for 10 weeks while 75 (25 individuals of each seed size) were not. After this growing period, each fertilized plant (n=75) was supplied with 100 mg of N. The experiment was established in a nursery, for 195 days, following a completely randomized design.

Plasticity and phenotypic change indices

Based on different seed sizes, plasticity indices (PI) were determined for phenotypic traits of seedlings when the fertilization factor was significant at 0.05, *i.e.*, fertilized seedlings. Plasticity indices were calculated following the Hernández-Pérez *et al.* (2001) equation to assess the association degree between the species' response to fertilization and its growth potential.

The indices of phenotypic change were determined based on the graphical method proposed by Pigliucci and Schlichting (1996), in which the average values of the phenotypic traits of seeds (of different sizes) are represented in a two-dimensional plane. The abscissa axis corresponds to unfertilized seedlings, while the ordinate axis corresponds to fertilizedseedlings.

Statistical analysis

Firstly, Mardia's multivariate normality test (H_0: The variables come from a multivariate normal distribution) (Mardia, 1974) was applied to the height, root collar diameter, root dry biomass, and stem dry biomass variables. As the statistic value was 36.0903 and the p-value was 0.0150, *i.e.*, less than the significance level (p-value<0.05), it was concluded that the data did not come from a multivariate normal distribution. Therefore, statistical analyses using non-parametric statistics was perform, specifically to determine the phenotypic traits that had a significant response to fertilizer addition. Mann-Whitney-Wilcoxon test and non-parametric multivariate analysis of variance were applied to determine the factors (Fertilization, seed size, and interaction) which affected the plant growth. All analyses were performed with R statistical software version 3.2.3. (R Core Team, 2015).

To assess the biomass allocation patterns, an allometric analysis of the changes between root dry biomass and stem dry biomass was performed. For this purpose, a regression model with indicator variables was used to determine whether the behavior of root dry biomass differed in both the intercept and slope as a function of stem dry biomass and fertilization. To obtain the test statistic and conclude based on the stated hypothesis, the extra sum of squares method was applied (Montgomery *et al.*, 2012).

To complement the interpretation of the results from the experimental design with a multivariate approach, a Canonical Discriminant Analysis was performed, which allowed to identify differences between groups (treatments), based on the characteristics (variables) measured on the individuals of these groups, and understand the relation between the variables within the groups. The latter was defined by the combinations between fertilization levels (fertilized and unfertilized seedlings) and seed size (small, medium, and large). With the Canonical Discriminant Analysis, canonical variables were obtained from the original variables (phenotypic traits), with which the separation and conformation of groups of individuals were achieved by maximizing the variance between groups and minimizing the variance within groups. Finally, Mahalanobis distances were calculated in order to know the groups.

RESULTS AND DISCUSION

The results show that the application of fertilizer on *Q. variabilis* seedlings produced from different seed sizes, promoted phenotypic and plasticity changes, as well as differences in their allocation patterns. This is because the species respond differently during growth and biomass production (stem and root) to the fertilization stimulus imposed (Agathokleous *et al.*, 2022; Pająk *et al.*, 2022), at an early age and independent of the germplasm size.

Fertilization in *Q. variabilis* seedlings significantly affected their morphological traits, according to the Mann-Whitney-Wilcoxon (W) test (Table 1), *i.e.*, the means of all morphological traits showed a significant difference (0.05) between fertilized and unfertilized seedlings, except for the root dry biomass trait. With regard to the non-parametric multivariate analysis, significant differences were found both in the fertilization (p-value=0.0001) and seed size (p-value=0.0001) factors and in the fertilization and seed size interaction (p-value=0.0001) in phenotypic traits height, root collar diameter, root dry biomass, and stem dry biomass. Fertilized seedlings showed higher growth, thus higher biomass production than unfertilized seedlings. Seedlings germinated from large and medium seeds obtained the highest values in height, root collar diameter, root dry biomass, and stem dry biomass compared to seedlings produced from the smaller seeds (Table 2).

Table 1. Averages of morphological attributes of *Quercus variabilis* seedlings.

Trait[†]	Test statistics (W)	p-value	Level[‡]	
			F	UF
H	1846.5	0.0002	31.7^{a}	27.8^{b}
D	1744.0	5.95×10^{-05}	3.5^{a}	3.2^{b}
RDB	2319.0	0.06379	5.1^{a}	4.7^{a}
SDB	1520.0	1.19×10^{-06}	0.9^{a}	0.7^{b}

[†] H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass (g·seedling^{-1}), SDB: Stem dry biomass (g·seedling^{-1}). [‡] F: Fertilized seedlings, UF: Unfertilized seedlings. Different letters in each line indicate significant differences (α=0.05) due to fertilization.

Table 2. Growth and biomass of *Q. variabilis* seedlings by seed size. Based on Student's t-test, different letters per column indicate significant differences (p-value=0.05) due to fertilization.

Seed size	Level[†]	H[‡]	D	RDB	SDB
Large	F	36.59^{a}	3.80^{a}	5.27^{a}	1.23^{a}
Large	UF	30.53^{b}	3.51^{b}	5.34^{a}	0.89^{b}
Medium	F	32.72^{a}	3.56^{a}	5.34^{a}	0.96^{a}
Medium	UF	30.74^{a}	3.29^{b}	5.19^{a}	0.78^{b}
Small	F	25.66^{a}	3.22^{a}	4.69^{a}	0.64^{a}
Small	UF	22.20^{b}	2.86^{b}	3.66^{b}	0.45^{b}

[†] F: Fertilized plant, UF: Unfertilized plant. [‡] H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass (g·plántula^{-1}), SDB: Stem dry biomass (g·seedling^{-1}).

The interaction between seed size and fertilization of *Q. variabilis* seedlings affected the growth and biomass variables. This variation occurred because fertilized seedlings tend to increase in size on providing nutrients (Dziedek *et al.*, 2017). Similar results in the growth have been reported in *Quercus suber* L. (Mechergui *et al.*, 2021), while biomass has been reported for *Quercus brantii* Lindl (Yadegari and Seyedi, 2019); both aspects are related to the amount of protein, carbohydrate, and lipid reserves because these promote the growth and biomass production (Mechergui *et al.*, 2021). It has been reported that genetic component plays an important role in the interaction between seed size and fertilization (Clark and Schlarbaum, 2018), which could have been the reason for observing differences in the interrelationship between these two factors.

Q. variabilis seedlings showed marked changes (plasticity) in all traits in response to the nutrient application, which highlighted the importance of fertilization in this species (Table 3). For most traits, small seeds showed the highest plasticity indices, while medium and large seeds showed the least (Table 3).

The changes in phenotypic indices three seed sizes were not distinct but showed changes in the analyzed traits (Figure 1). For the growth variables, medium seeds reached the highest values (0.94 for height and 0.92 for root collar diameter), while small seeds had the lowest values (0.86 for height and 0.88 for root collar diameter) (Figure 1a, b). Regarding

Table 3. Phenotypic plasticity indices of *Quercus variabilis* seedlings germinated from seeds of different sizes.

Trait†	Index		
	Small seed	**Medium seed**	**Large seed**
H	13.50	6.03	16.56
D	11.04	7.53	7.66
RDB	21.95	2.92	−1.44
SDB	29.78	18.56	27.36

†H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass (g·seedling^{-1}), SDB: Stem dry biomass (g·seedling^{-1}).

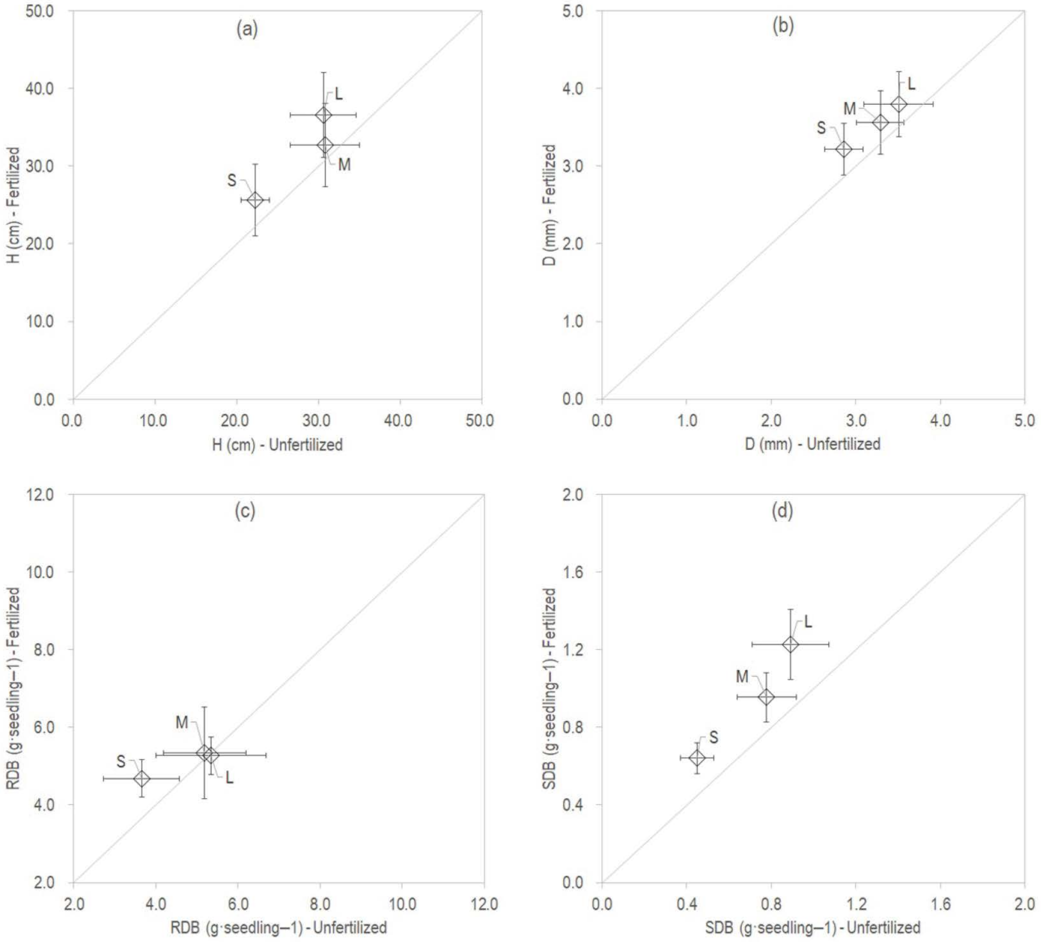

Figure 1. Plasticity analysis by seed size (S: small, M: medium and L: large) for growth variables and biomass components with a significant response to fertilization treatment in (a) H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass, SDB: Stem dry biomass. The bars indicate the standard errors in the two treatments.

biomass components, large seeds recorded the highest index for root dry biomass (1.01) (Figure 1c), while medium seeds had the highest values of plasticity for stem dry biomass (0.81) (Figure 1d).

For small seeds, growth plasticity indices showed higher values for diameter; however, in biomass-related plasticity indices higher values were obtained for all traits, although larger seeds showed higher values for height. These results varied from those reported in ten plant species (*Populus tremuloides* Michx., *Betula papyrifera* Marsh, *Betula alleghaniensis* Britton, *Acer saccharum* Marsh, *Larix laricina* (Du Roi) K. Koch, *Pinus banksiana* Lamb., *Pinus resinosa* Ait., *Pinus strobus* L., *Picea mariana* (Mill.) BSP and *Abies balsamea* (L.) Mill.) of North American trees (Walters and Reich, 2000), where high plasticity indices were observed in larger seeds in few analyzed variables. This can be attributed to the factors evaluated in the experiment (N content and seeding depth). The plasticity results showed that smaller seeds had higher growth and development capacity than medium and larger seeds, so they have a greater ecological advantage as they can be widely distributed at different sites (Moles and Westoby, 2004), *i.e.*, a higher value of root biomass indicates that seeds are more drought resistant (Olmo *et al.*, 2014) and may have a better survival rate at sites with low moisture availability. However, these results differ from those reported by Miniño *et al.* (2014), who elucidated that a large seed would give a larger seedling with a higher probability of survival as compared to a smaller one. For the stem dry biomass, *Q. variabilis* recorded lower values (18.56 to 29.78) compared to pine species (35.04 to 43.03) recorded by Flores *et al.* (2018) using the same phenotypic plasticity index.

According to the allometric analysis, the regression model showed differences in both intercept and slope, *i.e.*, the null hypothesis (H_0) was rejected at a 0.05 significance level. Comparing the regression coefficients of the generated models (root dry biomass=1.8374+4.0943*stem dry biomass and root dry biomass=3.4956+1.7038*stem dry biomass; unfertilized and fertilized seedlings, respectively), the expected root dry biomass was approximately 2.4-fold higher in unfertilized seedlings with respect to the fertilized seedlings. In other words, an increase of 1.7038 and 4.0943 units in root dry biomass is expected for each unit increase in stem dry biomass in fertilized and unfertilized seedlings, respectively.

Application of fertilizer on *Q. variabilis* seedlings promoted changes in biomass allocation, probably due to the capacity of the seedlings to alter their nutrient reserves by imposing stimulus (fertilization) during early stages of development (Bloom *et al.*, 1985). This condition showed that the fertilization routine applied affected biomass production, which is not always the case, *e.g.*, in a *Populus maximowiczii* A. Henry × *P. balsamifera* L. hybrid, no significant effect was noted on the biomass allocation. According to the specialization theory (Lortie and Aarssen, 1996), genotypes adapted to favorable conditions will perform better in benign environment, which was observed in *Q. variabilis* found at sites with good nutrient supply.

Non-parametric multivariate analysis of the data based on Canonical Discriminant Analysis revealed that the first two canonical components were significant (p-value<0.0002) and explained 98.80% of the total variability. The first canonical component explained 95.31%, where the main phenotypic traits were root dry biomass and stem dry biomass; while the second canonical component explained only 3.49%, and the most important phenotypic traits were root dry biomass and stem dry biomass (Table 4) by presenting the largest weights in both components. However, only the first canonical component was considered because it explained more than 95% of the total variability, and the most important phenotypic traits were root dry biomass and stem dry biomass in both components.

The analysis of the groups formed according to the weights of the first canonical component (Table 4) showed that groups 3 and 6 were characterized by high root dry biomass as the canonical component value was negative, and with less intensity, the groups 1 and 2 also were characterized by the root dry biomass. In contrast, groups 4 and 5 differed by presenting larger heights, diameters, and stem dry biomass and a small root dry biomass such that the value of the canonical component was positive (Table 4). As in the previous case, groups 1 and 2 presented a positive canonical component value (Table 3), this indicated that groups 1 and 2 were more unstable than the rest by presenting positive and negative values of the canonical component.

According to the results of the canonical discriminant analysis, the phenotypic traits of root dry biomass and stem dry biomass were the most important since they had the highest proportion of variability compared to the remaining attributes, so these variables were most important for grouping individuals. In this regard, it is to be expected —in young *Q. variabilis* seedlings— that during routine application of fertilization, the variables associated with biomass showed a greater response than those related to growth, *i.e.*, height and diameter. These results corroborate with those reported for root dry biomass and stem dry biomass in *Q. variabilis* (Li *et al.*, 2014; Wang *et al.*, 2016), and in *Agathis australis* (D. Don) Lindl., *Dacrycarpus dacrydiodes* (A. Rich.) de Laub., *Knightia excelsa* R. Br. and *Laurelia novae-zelandiae* A. Cunn (Kramer-Walter and Laughlin, 2017).

The root biomass is an important aspect of *Q. variabilis* since the characteristics associated with the root abundance and quality indicate that seedlings have greater survival rate (Grossnickle, 2012) and growth potential (Grossnickle and MacDonald, 2018) in fields.

Also, the canonical dispersion plot showed a marked difference between fertilized and unfertilized seedlings; also, unfertilized and seedlings produced from medium and smaller seeds presented a more heterogeneous behavior than the remaining ones. This implies that for *Q. variabilis*, the variation is influenced by seed size, which could be related to the number of nutritional reserves accumulated in the seeds, as is the case of *Q. glauca* Thunb. (Negi and Rawal, 2018).

Table 4. Total explained variance percentage and values of canonical coefficients.

Variable†	**Canonical components**	
	CC1 (95.31%)	**CC2 (3.49%)**
H	0.31140	0.41100
D	0.17537	0.12674
RDB	−0.43547	1.35510
SDB	2.15264	−1.12208

†H: Height (cm), D: Root collar diameter (mm), RDB: Root dry biomass (g·seedling^{-1}), SDB: Stem dry biomass (g·seedling^{-1}).

According to the Mahalanobis distances, most of the distances were significant at p-value$<$0.05 because there were differences between groups in the lower triangular matrix (Table 5) and p-values$<$0.05 (upper triangular matrix). It was possible to identify the different groups defined between fertilized and unfertilized seedlings, which were produced in different seed sizes (small, medium and large), except for the distance between the groups defined by unfertilized seedlings produced with larger seeds (Group 1) and fertilized seedlings produced with medium seed (Group 5) (p-value$=$0.2442).

Table 5. Mahalanobis distance matrix between groups defined by fertilized and unfertilized seedlings produced with three seed sizes of *Quercus variabilis*.

Group	**Group†**					
	1	**2**	**3**	**4**	**5**	**6**
1	0	0.0087	0.0001	0.0001	0.2131	0.0001
2	1.157*	0	0.0001	0.0001	0.0001	0.0001
3	14.9725*	9.430*	0	0.0001	0.0001	0.0001
4	8.156*	13.851*	41.671*	0	0.0001	0.0001
5	0.4811ns	2.406*	19.541*	4.875*	0	0.0001
6	4.123*	1.983*	3.478*	23.006*	7.130*	0

†1: Non-fertilized plant with large seed, 2: non-fertilized plant with medium seed, 3: non-fertilized plant with small seed, 4: fertilized plant with large seed, 5: fertilized plant with medium seed and 6: fertilized plant with small seed. *: Significant difference (p-value$\leq$0.05), ns: non-significant difference.

CONCLUSIONS

The above results showed that phenotypic plasticity in *Q. variabilis* was observed between fertilized and unfertilized seedlings when they were produced from small, medium, and large seeds. It is suggested to use medium and large seeds during nursery plant production and to establish fertilized plants in the field because they may have a greater advantage in survival and growth than unfertilized seedlings.

ACKNOWLEDGMENTS

The authors thank the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) for financing publishing this research, and the reviewers of the manuscript for their comments.

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