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Growth response of tissue cultured-derived bamboo (*Bambusa tulda Roxb.*) plantlets to sources and levels of nitrogen

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ABSTRACT

Information on nitrogen nutrition of bamboo plants during the nursery stage is very limited. The study was conducted to generate more information regarding the nitrogen nutrition of bamboo (*Bambusa tulda* Roxb.) specifically to evaluate the effect of forms and levels of N on the early growth stage performance of tissue culture-derived bamboo plantlets and identify the best form and optimum level of nitrogen for bamboo plants during the nursery stage.

The acclimatized tissue culture-derived bamboo (*Bambusa tulda* Rox.) plantlets were grown in black polyethylene bags measuring 24x15cm containing potting medium composed of 1:1v/v mixture of garden soil and rice hull charcoal. These were applied with two forms of nitrogen (N_1-nitrate-N and N_2-ammonium-N) which served as the factor A and 3 levels of nitrogen (L_1-0g N plant^{-1}, L_2-0.25g N plant^{-1}, and L_3-0.50g N plant^{-1} which was the factor B of a 2x3 factorial RCBD experiment. The effects of the treatments on the performance of the bamboo plantlets were evaluated by gathering growth parameters such as height, number of leaves and culms and leaf size, and dry weights of leaf, stem, root, and total biomass weight.

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The form of N applied did not significantly influenced the size of bamboo plants but significantly affected the plant dry weight. Plants fertilized with ammonium-N produced heavier biomass weight than those applied with nitrate-N. The levels of N-application significantly influenced both the plant size and weight. Application of 0.25g N and 0.5g N plant^{-1} significantly increased the number of culms and leaves and total plant biomass dry weight. Based on the effect on the size and weight of plants, application of 0.25g N plant^{-1} was already optimum for bamboo plants during the nursery stage. For bamboo plants at the nursery stage, ammonium-N was better than nitrate-N with 0.25g N plant^{-1} as the optimum level of application.

Keywords: Nitrogen sources, bamboo plantlets, growth, biomass

INTRODUCTION

Bamboos of the family Poaceae and sub-family *Bambusoideae* are comprised of more than 115 genera and 1,400 species and are considered one of the most utilized and versatile plants on Earth. They are one of the fastest-growing plants in the world and are the largest and tallest plants in the grass family (Cho et al 2011). Bamboos are used for a diverse range of applications in the paper and handicraft industries, in house construction and in making furniture, water pipes and storage vessels as well as for landscaping, ornamental display and a thousand other uses (Nongdam & Tikendra 2014).

In addition to these multiple non-food applications, the juvenile bamboo shoots, which are low in fat but high in protein, amino acids, minerals, fibre and carbohydrates is a widely acclaimed nutrient rich food item (Nongdam & Tikendra 2014). Commercial planting of bamboos is considered desirable because of their high economic and ecological potentials (Meniano & Abella 2018).

One important requirement for successful bamboo cultivation is the availability of good quality planting materials of a desirable species. Bamboos can be propagated either by seeds, culm cuttings, branch cuttings or marcotting, offset or rhizome cutting and tissue culture (Roxas nd).

For mass propagation of disease-free bamboo planting materials, the use of the tissue culture technique has been reported to be very promising (Zamora et al 1992). A very important component of any effective tissue culture protocol for bamboo is the effective raising of the regenerated seedlings/plantlets until achieving plantable size. The plantlets, after their hardening and acclimatization, are planted in pots of various sizes which may contain different potting mixes. To sustain the nutrient supply, fertilizer must be applied. Among the plant nutrients, bamboo requires nitrogen (N), phosphorus (P) and potassium (K) with N being required in high amounts (Thomas 1988, Maoyi et al 1988, Gao et al 2016). Application of 0.25g complete (15-15-15) fertilizer/plant at potting was recommended (UNIDO 2009). Moreover, Thomas (1988) reported that application of 40g N, 27g P and 25g K per pot significantly boosted the growth and biomass production of *B. arundinacea* seedlings raised in pots.

Despite the importance of fertilizer application for bamboo plantlets during the nursery stage, information regarding the fertilizer application, in particular the appropriate level and the best source of nitrogen for bamboo plantlets is very limited. This study aimed to evaluate the effect of the forms of inorganic N on the growth performance of tissue culture-derived bamboo plantlets; to assess the effect

of varying N levels on the performance of tissue culture-derived bamboo plantlets; and to identify the best source and optimum level of N application for bamboo plantlets during the nursery stage.

MATERIALS AND METHODS

Preparation of the Polyethylene Bags and Potting Medium

Black polyethylene bags measuring 24cmx15cm and perforated with four drainage holes were used to contain the medium. Potting medium was prepared by mixing equal parts (v/v) sterilized garden soil and rice hull charcoal. The bags were filled with equal amounts of potting medium.

Preparation of Plantlets and Bagging

Fully hardened tissue culture-derived bamboo (*Bambusa tulda* Roxb.) plantlets gradually exposed to natural environmental conditions and planted in sterilized soil medium were obtained from the Plant Tissue Culture Laboratory of the Department of Horticulture, College of Agriculture and Food Science, Visayas State University, Baybay, Leyte. Plantlets with more or less uniform size and vigor were selected. The plantlets were carefully removed from their small plastic bags and then re-bagged into the prepared (24cmx15cm) bags by burying the base/roots in the potting medium followed by slight pressing of the medium around the base to ensure good root and potting medium contact.

Experimental Design and Treatments

The experiment was laid out in a 2x3 factorial randomized complete block design (RCBD) with three replications and having 5 sample plantlets per treatment per replicate. The form of Nitrogen (N_1-Calcium nitrate and N_2-Ammonium sulphate) was assigned as Factor A and the three levels of Nitrogen application (L_1) – (0g N + 0.5g P_2O_5 + 0.5g K_2O/plantlet [2.5g Solophos/plantlet + 0.83g KCL/plantlet]); (L_2) – (0.25g N + 0.5g P_2O_5 + 0.5g K_2O/plantlet [1.61g Calcium nitrate or 1.19g Ammosul /plantlet] + 2.5g Solophos/plantlet + 0.83g KCL/plantlet) and (L_3) – (0.50g N + 0.5g P_2O_5 + 0.5g K_2O/plantlet [3.22g calcium nitrate or 2.38 Ammosul] + 2.5g Solophos/plantlet + 0.83g KCL/plantlet]) as Factor B, respectively.

Fertilizer Applications

Calcium nitrate (15.5-0-0) and Ammosul (21-0-0) were used as sources of nitrogen while Solophos (0-20-0) and Muriate of potash (0-0-60) were used as sources of P and K. The amounts of each fertilizer material as shown above were computed based on the applied rates and the fertilizer grade of the fertilizer materials. N (0, control, 0.25 and 0.50g plantlet^{-1}), P_2O_5 (0.5g plantlet^{-1}) and K_2O (0.5g plantlet^{-1}).The whole amounts of P and K, and half the amount of N were applied 1 month after bagging.The remaining half amount of N was applied 1 month after the first application. The Solophos and Muriate of potash (L_1) and Calcium nitrate or Ammosul, Solphos and Muriate of potash (L_2 and L_3) allocated to each plantlet were mixed thoroughly, placed inside three holes made around the base of the plantlet and then immediately covered with the potting medium.

Care and Maintenance of the Experimental Plants

The experimental plantlets were placed inside a screenhouse and were arranged in a 2x3 Factorial RCBD lay-out.The potted plantlets were arranged with a 25cm distance center to center. To prevent plantlets from getting nutrients from the soil, each bag was placed on top of a piece of plastic sheet which prevented the roots having direct contact with the soil. Watering and removal of weeds were done regularly.The plantlets were sprayed with insecticide (Cypermethrin 25 EC solution at 0.5mL^{-1} liter) twice to control leaf eating and sucking insects.

Data Gathered and Statistical Analysis

Growth parameters such as plant height (cm), number of culms, and the number and size (length and width) of the leaves were measured 3 months after potting. Plant dry weight (biomass) was determined 4 months after potting. All five sample plants in each treatment and replication were used in data gathering. For biomass determination, the plants were carefully removed from each bag by dipping the bag/plants in a pail of water and then after the medium had been saturated this was pressed lightly to loosen it. Complete cleaning of the roots was done by washing the roots in running water. The clean plants were separated into sections comprising the leaves, stem and roots. The samples were placed in properly labeled paper bags and were oven dried at 70°C for 72h or until constant weight was attained. After cooling, the weights were measured using a digital weighing scale.

The data gathered were statistically analyzed using the computer software Statistical Tool for Agricultural Research (STAR) version 2.0.1. The presence of significant differences among treatments was determined using ANOVA in factorial Randomized Complete Block Design. Mean separation was done using Tukey's HSD at 5% level of significance.

RESULTS AND DISCUSSION

Effect on Plant Size

The growth parameters of the bamboo plants measured in this study were height, number of culms, and number and size of leaves. None of the plant size parameters were significantly influenced by the sources of nitrogen. Based on plant response in terms of size, the results indicated that both the nitrate and ammonium form of nitrogen had a comparable effect on the growth parameters of bamboo plants during the first 3 months from potting.

In contrast to the effect of N-source on plantlet size, all the growth parameters of bamboo plantlets evaluated were significantly influenced by the level of nitrogen fertilization except for the height (Table 1). Plants applied with two levels of nitrogen produced more culms than the non-N fertilized control plants. The number of culms on plants that received either 0.25g N or 0.50g N plant^{-1} were comparable with each other. The number of leaves on plants given 0.50g N plant^{-1} was significantly higher than those given 0.25g N plant^{-1},which had a number of leaves comparable to plants that were not given any nitrogen fertilizer.

Table 1. Size of of bamboo plants as influenced by sources and levels of nitrogen 3 months after bagging

Treatment	Plant Height (cm)	Number of Culms plant^{-1}	Number of Leaves plant^{-1}	Length of Leaves (cm)	Width of Leaves (cm)
Factor A-Form of N					
N_1–Nitrate	54.82a	16.22a	109.40a	14.02a	2.38a
N_2–Ammonium	51.67a	14.78a	103.38a	13.76a	2.40a
Factor B - Level of N (g plant^{-1})					
L_1–0g	53.13a	12.83b	89.70b	13.85ab	2.34ab
L_2–0.25g	55.52a	16.22a	104.33b	15.56a	2.72a
L_3–0.50g	51.08a	17.47a	125.13a	12.27b	2.10b
CV (%)	10.10	11.28	9.94	10.98	15.15

Means in a N source and N levels column having the same letter are not significantly different at 5% level based on Tukey HSD.

Furthermore, plants given the highest level of N (0.5g plant^{-1}) had shorter and narrower leaves compared with the leaves of plants applied with 0.25g N plant^{-1} and the control plants. Smaller leaves among plants that received higher level of N was attributed to the adverse effect of the salt stress suffered by plants that resulted in leaf burning and hence leaf growth reduction. The deleterious effects of salinity affect different physiological and metabolic processes of plants (Parida & Das 2005). Plants exposed to salinity experience water stress, which in turn reduces leaf expansion (Carillo et al 2011). Salinity stress strongly reduced shoot and root length of bamboo *Dendrocalamus strictus* and *Dendrocalamus longispathus* (Pulavarty & Sarangi 2015) and significantly reduced leaf area of black rice (Nissa et al 2022) and corn (El Sayed 2011). The favorable effect of the application of the appropriate level of nitrogen on culm and leaf production confirmed earlier reports (Marie 2016, UNID0 2009, Kim et al 2018) that indicated the responsiveness of bamboo to nitrogen fertilization.

Effect on Biomass Weight

The biomass weight reflects the amount of stored food which has significant influence in the survival of the bamboo plantlets once outplanted in the field. The dry weights of the three parts of the bamboo plant and the total plant dry weights were all affected by both forms and levels of nitrogen application (Figure 1).

Regardless of the levels of N, plants applied with ammonium N had significantly heavier dry weights of leaves, stem, roots and hence the total plant dry weights compared to plants applied with nitrate N. While the size of the plants applied with two forms of N were comparable (Table 1), their weights significantly differed (Figure 1), with plants receiving ammonium-N having heavier biomass weight than those applied with nitrate-N. The result suggests that the heavier biomass weight among ammonium-Ntreated plants was probably due to the effectiveness of ammonium-N in enhancing dry matter production and accumulation rather than by increasing plant size. Spratt and Gasser (1970), found that wheat applied with ammonium-N had a heavier weight of leaves and stems during early growth than those applied with nitrate-N. Higher shoot and total biomass weight in plants receiving ammonium-N over those applied with nitrate-N were also reported in *Araucaria angustifolia* L. (Garben & Dillenburg 2008), Douglas fir, Sitka spruce, and white spruce (Van Den Driessche 1971) and in tea (Sarwar et al 2007).

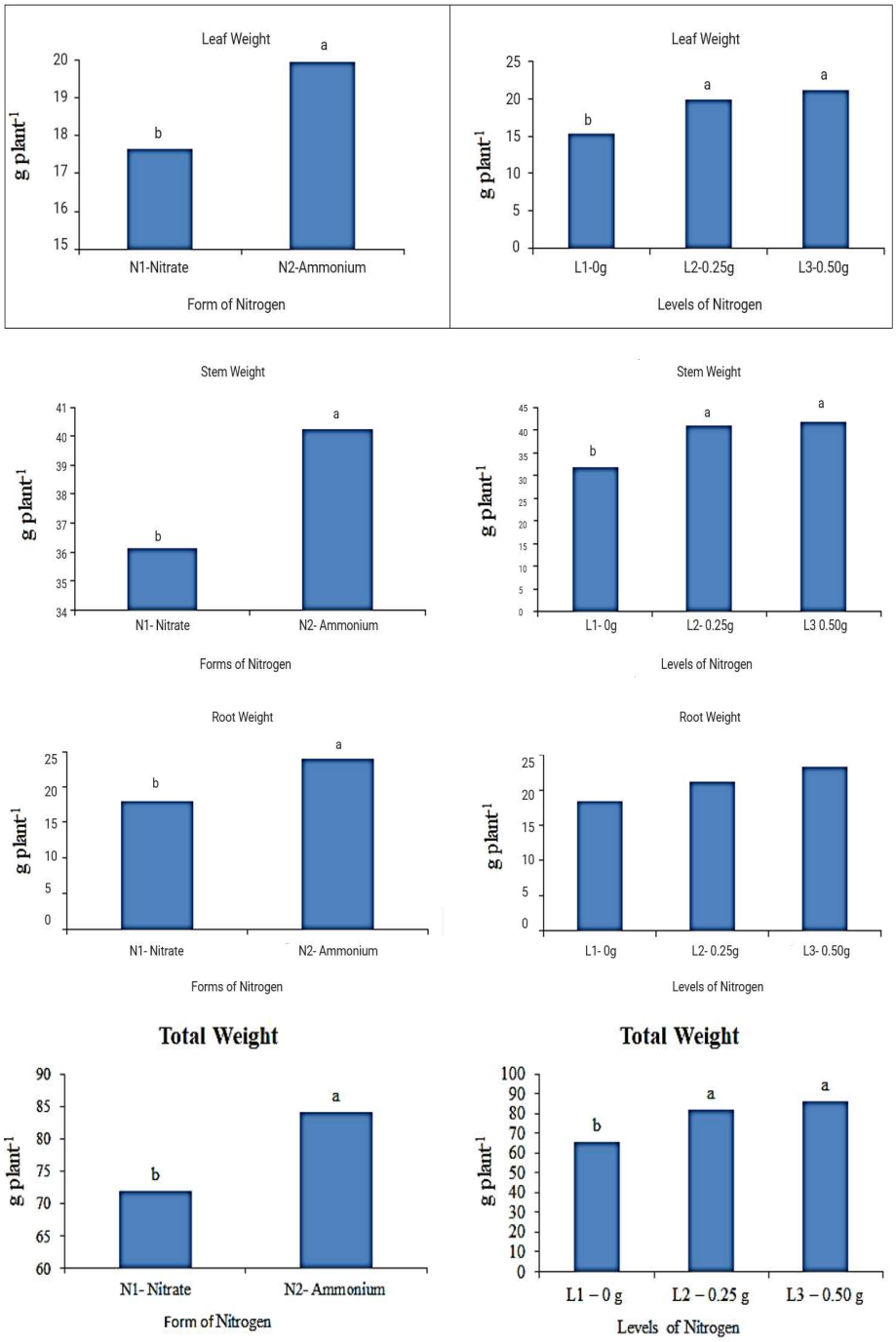

Figure 1. Dry weights of different parts of the bamboo plants and total biomass as influenced by forms and levels of nitrogen sampled 4 months after potting

Regardless of the forms of N, plants applied with 0.25 and 0.5g N plant^{-1} had significantly heavier leaf, stem, and total plant dry weights than plants that did not receive nitrogen fertilizer. Biomass weight between plants receiving 0.25g N and 0.5g N plant^{-1} were comparable suggesting that the 0.25g N could already be the optimum level. The heavier biomass weight among N-fertilized plants was attributed to the production of more culms and leaves (Table 1).

CONCLUSION

Based from the results of the experiment, the form of N applied did not significantly influence the size of bamboo plants, but significantly affected the plant dry weight with plants fertilized with ammonium-N producing heavier biomass than those applied with nitrate-N. The levels of N application significantly influenced the size and biomass weight of the bamboo plants. Application of 0.25g N and 0.5g N plant^{-1} significantly increased the number of culms and leaves and total plant dry weight. The size and weight of plants applied with the two levels of N were comparable suggesting that 0.25g N plant^{-1} was already optimum for bamboo plants during the nursery stage. For bamboo plants, ammonium-N was better than nitrate-N and 0.25g N plant^{-1} was the optimum level.

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AUTHOR CONTRIBUTIONS

Substantial contributions to the conception or design of the work; analysis or interpretation of data; drafting the work or critically reviewing it; and final approval of the version to be published.

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Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Data and materials generated or analyzed during the study are included in this article and its supplementary files. They are also available from the corresponding author upon request.

ETHICAL CONSIDERATIONS

Not applicable.

COMPETING INTEREST

The authors declare that they have no conflict of interests.

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