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Vegetative propagation of bird's-foot trefoil (*Lotus corniculatus* L.) using different rooting agents

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ABSTRACT

Objective: To evaluate the growth dynamics based on the dry matter production of *Lotus corniculatus* L. variety 202700 and its morphological composition.

Design/Methodology/Approach: The study was carried out in greenhouses conditions, from December 2020 to May 2021. The effect of indole butyric acid (IBA) growth promoter in solid Radix 1500 and liquid Radix T 3000 presentation (S+RSolid and S+RLiquid) and a control (Substrate) was evaluated. A completely randomized design with three replications was used, each with twenty pots as replications. 1300 pots were planted, of which 20 were taken monthly from each treatment (30, 60, 90, 120 and 150 days) for subsequent data recording.

Results: S+RSolid was the one that presented the highest values followed by S+RLiquid and Control (S) respectively.

Study Limitations/Implications: Destructive sampling of less than 30 days or greater than 150 days was not considered.

Findings/Conclusions: The plants with the application of 1500 ppm of IBA registered the greatest response in terms of variables: number of leaves, number of stems, leaves (g), stems (g), roots (g), root height (cm), root volume (cm^3), greenness index, leaf area (cm^2/g) and plant height (cm), with respect to the rest of the treatments.

Keywords: *Lotus corniculatus* L., *Medicago sativa* L., *Trifolium repens* L., *Trifolium pratense* L.

Image by beauty of nature at Pixabay

INTRODUCTION

Bird's-foot trefoil (*Lotus corniculatus* L.,) is a Fabaceae plant, with relevant fodder potential (De los Santos and Steiner, 2003); it represents 90% of the area sown within the genus worldwide, mainly United States, Australia, Chile and Argentina (Escaray *et al.*, 2012). It presents adaptive qualities to diverse soil textures (Garcia, 2011), it also tolerates flooding, drought, saline soils, acidity, low fertility and phosphorus, and in associations it improves nitrogen availability (Striker *et al.*, 2005). It thrives under conditions where alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) are unlikely to establish (Grant, 2009). Kirkbride (1999) mentions that Bird's-foot trefoil is a very variable species (it is taxonomically classified in different ways) and the most widely distributed of the *Lotus* varieties in the world. In terms of nutritional quality, it is mentioned that it is similar to the most common temperate legumes such as alfalfa, white clover and red clover for its excellent quality protein, which is 17.7 to 21.6% DM (Marley *et al.*, 2006). It contains condensed tannins that prevent timpanism in ruminants and protect protein degradation in the rumen (Ayala and Carámbula, 2009). *L. corniculatus* is an important option for livestock feed, it grows best in cold and humid climates, although also in tropical and subtropical climates. Like all fodders, it has marked disadvantages, such as slow initial establishment and low persistence (Ixtaina and Mujica, 2010).

Productivity and morphological and physiological characteristics of crops are modified by nutritional, genetic or specific factors such as regulators of growth development (Cortes *et al.*, 2019).

In the particular case of Mexico, there is little or no information about the behavior of different fodder ecotypes and the application of growth regulators. Growth regulators applied as rooting agents are used to induce and stimulate root development and stem thickening, through the content of nutrients and phytohormones that intervene in specific physiological processes, which, if not present in the plant make it difficult to achieve satisfactory development (Garay *et al.*, 2008). Indoleacetic acid and tryptophan (hormone and precursor) promote root cell division and plant growth (Chilon, E. and Chilon, J., 2015). Castrillon *et al.* (2008) studied the effects of indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and 1-naphthaleneacetic acid (NAA) on the survival of *Pushgay* cuttings in soil + peat and peat only, at different concentrations and showed that IBA at 200 mg/L in soil + peat presented the longest survival time, with 43 days; meanwhile, without regulator the cuttings survived only 21 days. Therefore, the objective of this study was to evaluate the effect of indolbutyric acid in liquid and solid form (RADIX®) on the rooting of bird's-foot trefoil stems in greenhouses.

MATERIALS AND METHODS

Location and date

The research was carried out in a glass greenhouse with passive ventilation by means of manually opened lateral vents at Colegio de Postgraduados, Campus Montecillo, Estado de México, located at an altitude of 2,250 masl. According to the Köppen's climate classification modified by García (2004), the region is C (W0) (W) b (i´) g, which corresponds to a temperate subhumid climate with summer rains and dry season in the winter, with low thermal fluctuation, mean annual rainfall of 686 mm and a mean annual temperature of 15.9 °C, with the month of May as the warmest and January as the coldest.

Plant matter

Original seed of genotype 202700 bird's-foot trefoil germplasm of erect habit originally from Uruguay was used, which was obtained through the Plant Genetic Resources Management Program at Colegio de Postgraduados, from the United States Department of Agriculture (USDA-ARS), Beltsville, Maryland, USA; this was evaluated for the first time since 1997, in an adaptability trial (García *et al.*, 2015; Álvarez *et al.*, 2018), but because the plants sown in the field have not generated seed they continue to be propagated by cuttings taken from the crown of those plants.

Treatments and experimental unit

The experiment was established in a completely randomized design (CRD), with three treatments, each with twenty pots as replications, every 30 days for 150 days.

Handling of potted plants

The substrate used was a mixture of agrolite (35.48%), peat moss (35.48%), vermiculite (3.22%), and soil (25.80%); a 17×17 cm bag was used (400 caliber with a capacity of 0.95 liters), which was filled with the mixture of substrates, irrigated at field capacity. The transplant began manually immediately, impregnating the rooting agent at 3 cm from the base two stems with the corresponding concentration and presentation; they were planted 4 cm deep in the pot, the distance between pots was separated 1 cm by treatments, watering was done weekly, and Captan® 50 was used to avoid root disease problems while no fertilization was done.

The treatments applied were the control, which consisted of substrate (S) without rooting agent; treatment two was substrate + solid rooting agent RADIX® 1500 (S+RSolid); and treatment three was substrate + liquid rooting agent RADIX® T 3000 (S+RLiquid). The experiment was established in December 2020 to April 2021. In the experiment, 1300 pots were used (to have enough repetitions for the experiment) in which cuttings from crowns of mother plants established in the field were transplanted (Garcia *et al.*, 2015; Alvarez *et al.*, 2018). The evaluations were carried out for 5 months, applying the destructive method, to observe the growth dynamics of the root and aerial part of the bird's-foot trefoil. The first sampling was performed at 30 days and, subsequently, cuttings were scheduled every 30 days, until reaching the age of 150 days (5 months).

Variables evaluated

Morphological composition

To determine the morphological composition, fodder harvested from the pots was separated into its components: leaves, stems, roots and dead material. They were placed in brown Kraft paper bags and weighed individually in a digital scale with a capacity of 500 g and an approximation of 0.01 g. They were then placed in a forced air furnace at a temperature of 55 °C for 72 hours to obtain the dry weight.

Plant height (PH)

A graduated ruler of 50 cm length and 1 mm of precision was used. The plant was separated from the pot and the ruler was placed from the root to the base of the stem and then from the base of the stem to the last leaf on the stem (top of the leaflets).

Leaf:stem ratio

The data originated from the morphological composition (leaf and stem) of the trefoil plants were used to estimate the leaf:stem ratio, which was calculated using the following formula:

$$L{:}\ S = LS$$

Where: L: S=Leaf: stem ratio. H=Dry weight of leaf component (g DM pot^{-1}); divided by T=Dry weight of stem component (g Ms pot^{-1}).

Greenness index (GI)

Chlorophyll readings were taken with the Minolta SPAD 502, designed for fast non-destructive determination, which evaluates quantitatively the intensity of leaf greenness (650 to 940 nm), obtaining averages (3 measurements of three upper leaves) of 20 plants per treatment. Chlorophyll concentration (μmol of chlorophyll per m^2 of leaf and in SPAD or CCI units) is an indirect indication of plant health and condition, and non-destructive sampling allows monitoring chlorophyll concentration in plants during the experiment (Sainz and Echeverría, 1998).

Root volume (RV)

The values were recorded by water volume difference with a 250 ml graduated cylinder which was filled to its capacity and the roots of 20 previously washed and dried plants were introduced.

Leaf area (LA)

In each treatment, trefoil stems were cut at pot level, separated into stem and leaves, which were placed on acetates and immediately taken to the CID, Inc. leaf area integrator, model CI-202 scanner, from which leaf area readings were obtained in cm^2.

Statistical analysis

An analysis of variance was performed with the GLM procedure of the Statistical Analysis System (SAS, 1999), under a completely randomized design with 3 treatments and 20 replications, the means comparison for each dependent variable with increasing age was performed using Tukey's test ($P<0.05$) and a regression analysis for each variable in order to describe the trend, coefficient of determination and significance.

Greenhouse temperature

The temperature inside the greenhouse was obtained with a digital maximum and minimum thermometer (BioTemp) placed at 1.60 meters from ground level next to the plant pots. The monthly maximum temperature ranged between 22 °C and 45 °C and the monthly minimum temperature ranged between 8 °C and 14 °C.

RESULTS AND DISCUSSION

Morphological composition

When analyzing the behavior of the variables evaluated during the establishment (Table 1), it was found that there were significant differences between treatments. The means comparison in number of leaves (NLeaves), number of stems (NStems), leaves, stems and roots of *Lotus corniculatus* L. at different ages, showed that the variables increased steadily and had statistical differences ($p<0.05$). The S+RSolid at 150 days of age showed the highest value of these variables, followed by the Control (S) and S+RLiquid treatments with no differences between them. This shows that in the case of the regulators, the best results were obtained with Radix 1500 (Solid), since it favored the number of stems, number of leaves, and weight of roots, stems and leaves (Table 1 and Figure 1) from the beginning to the end of the experiment. This could be due to the difference in the impregnation of the products, with powder being more favorable as it is released gradually on contact with the moisture of the substrate than in liquid and the latter being more concentrated. The regression models and coefficients of determination were high ($R^2>0.86$) for the variables evaluated; the best fitting model was the polynomial model (Figures 1 and 2) for the treatments.

Table 1. Statistical significance of means for morphological variables in *Lotus corniculatus* L.

DDTE (days)	Treatment	NLeaves	NStems	Leaves (g pot^{-1})	Stems (g pot^{-1})	Roots (g pot^{-1})
30	Control (S)	38 b	3 c	0.01 c	0.02 b	0.08 b
	S+ESolid	66 a	6 a	0.04 a	0.06 a	0.26 a
	S+ELiquid	45 b	4 b	0.03 b	0.05 a	0.10 b
60	Control (S)	125 ab	3 b	0.08 b	0.05 b	0.12 b
	S+ESolid	152 a	4 a	0.13 a	0.10 a	0.33 a
	S+ELiquid	116 b	3 b	0.08 b	0.06 b	0.15 b
90	Control (S)	171 b	6 b	0.13 b	0.07 b	0.18 b
	S+ESolid	334 a	9 a	0.35 a	0.21 a	0.46 a
	S+ELiquid	132 b	4 c	0.12 b	0.07 b	0.14 b
120	Control (S)	371 b	10 b	0.27 b	0.17 b	0.31 b
	S+ESolid	811 a	19 a	0.61 a	0.41 a	0.66 a
	S+ELiquid	390 b	9 c	0.30 b	0.17 b	0.28 b
150	Control (S)	581 c	13 c	0.51 c	0.26 c	0.42 c
	S+ESolid	1867 a	30 a	1.43 a	1.15 a	1.73 a
	S+ELiquid	858 b	15 b	0.74 b	0.50 b	0.76 b
	DMS	148	2	0.12	0.09	0.12

*a, b, c.=Different letters in the same column are statistically different. Tukey at 0.05. NLeaves=Number of leaves; NStems=Number of stems; Control (S)=Substrate without rooter; S+ESolid=Substrate + solid rooter; S+ELiquid=Substrate + liquid rooter DDTE=Days after cutting transplant; DMS: Minimum significant difference.

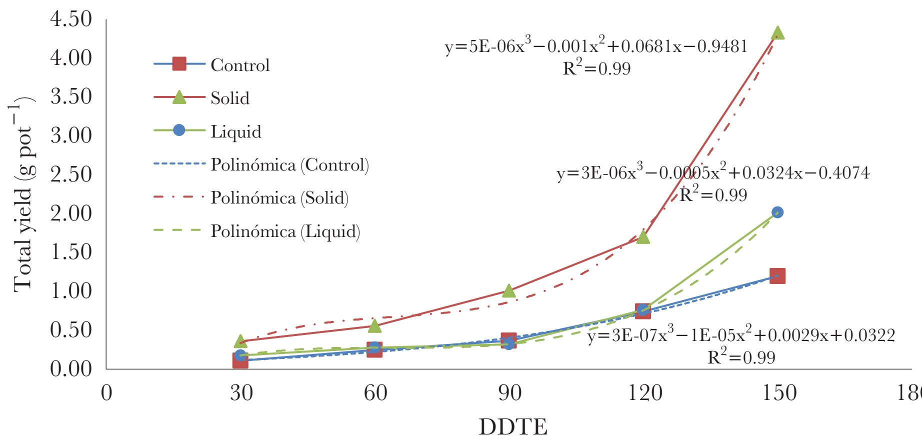

Figure 1. Average yield (g pot^{-1}) of *Lotus corniculatus* variety 202700, in plants 30 and 150 days after cutting transplant (DACT).

Plant height

Table 2 and Figure 2 show that there were differences between treatments, being S+RSolid with 29 cm the one that presented the highest values, followed by S+RLiquid with 22 cm and Control (S) with 20 cm, respectively, at 150 days. Garcia *et al.* (2015)

Table 2. Statistical significance of means for the variables leaf:stem ratio, root height, root volume, greenness index, leaf area and plant height in *Lotus corniculatus* L.

DDTE (days)	Treatment	H: T (g pot^{-1})	LR (cm)	Vol. (cm^3)	IV (μmol m^2)	AFE (cm^2/g)	AP (cm)
30	Control (S)	0.10 a	5.03 b	0.37 b	21.41 b	2.18 c	7.96 c
	S+ESolid	0.97 a	6.56 b	1.54 a	29.95 a	8.00 a	12.40 a
	S+ELiquid	0.86 a	10.26 a	1.57 a	24.55 b	5.32 b	10.52 b
60	Control (S)	1.80 a	18.09 a	0.94 b	30.47 ab	17.21 c	14.13 b
	S+ESolid	1.50 a	21.33 a	2.11 a	32.13 a	50.75 a	18.68 a
	S+ELiquid	1.33 a	17.17 a	1.94 a	26.13 b	43.24 b	14.64 b
90	Control (S)	2.27 a	22.81 b	2.66 b	28.26 c	22.70 b	15.24 b
	S+ESolid	1.69 a	32.29 a	5.84 a	38.47 a	61.35 a	19.93 a
	S+ELiquid	2.12 a	24.75 b	2.69 b	32.11 b	20.74 b	15.88 b
120	Control (S)	1.62 ab	33.81 b	6.13 b	27.85 b	38.03 b	16.28 c
	S+ESolid	1.50 b	38.60 a	14.18 a	34.75 a	90.02 a	21.81 a
	S+ELiquid	1.80 a	33.97 b	7.51 b	32.43 ab	45.31 b	17.51 b
150	Control (S)	1.97 a	30.27 b	9.19 c	41.19 b	46.53 c	20.22 c
	S+ESolid	1.24 c	37.47 a	27.71 a	45.79 a	252.65 a	29.49 a
	S+ELiquid	1.48 b	34.62 ab	15.69 b	41.51 b	115.97 b	21.85 b
	DMS	0.23	4.08	2.46	2.71	18.41	1.54

*a, b, c.=Different letters in the same column are statistically different. Tukey at 0.05. H: T=Leaf-Stem Relationship; LR=root length; Vol.=Root volume; Green index=Chlorophyll; AFE=Specific leaf area; AP=Plant height; Control S=Substrate without rooter; S+E Solid=Substrate + solid rooter; S+E Liquid= Substrate + liquid rooter; DDTE=Days after cutting transplant; DMS: Minimum significant difference.

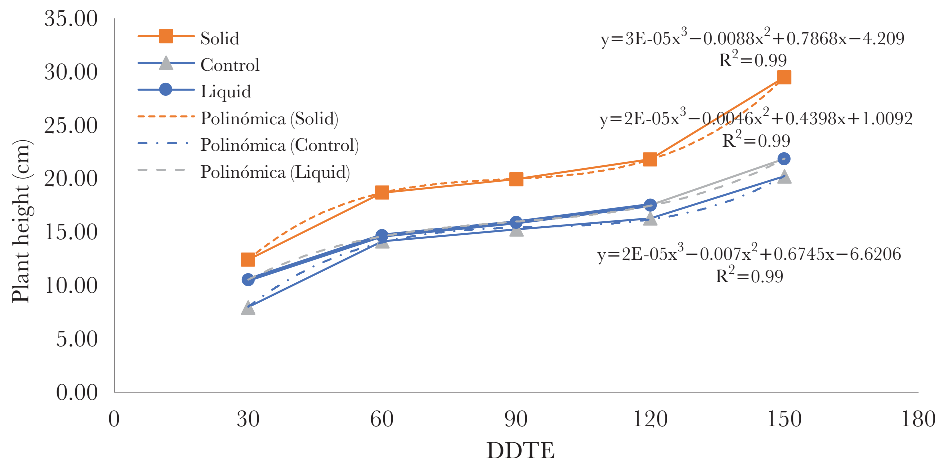

Figure 2. Average plant height (cm) of *Lotus corniculatus* variety 202700, in plants 30 and 150 days after cutting transplant (DACT).

mention that in the case of *Lotus corniculatus*, yield and height have also been related to erect and prostrate growth habits.

Leaf:stem ratio

When treatments were compared for the average leaf:stem ratio, no significant difference was found during the first 120 days, and it was not until 150 days when the Control (S) obtained the highest values, followed by S+RLiquid and S+RSolid (Table 2). The leaf:stem ratio values found were similar to those found in other studies. Berroterán (1989) obtained 2.02 in *Andropogon gayanus* and 0.61 in *Digitaria swazilandensis*; Calzada *et al*. (2014) obtained on average 0.73 in Maralfalfa grass (*Pennisetum* sp.), and the authors also mention that the greater the plant height, the greater the proportion of shaded leaves. Plant height presents a negative correlation with leaf biomass as plant height increases.

Greenness index

Regarding leaf greenness measured with the SPAD, the S+RSolid presented higher values than the S+RLiquid and Control (S) (Table 2). Wolfe *et al.* (1988) and Dwyer and Houwing (1991) mention that the chlorophyll content in the corn leaf is closely and positively related to the concentration of N in the leaf, and therefore, it reflects the nitrogenous state of the crop and the availability of N.

Root volume

Regarding root volume, it can be seen that it increased as the days of growth progressed (30-150 days) with the highest values for S+RSolid being 14 cm^3 followed by S+RLiquid 8 cm^3 and 6 cm^3 for control (S) at 120 days; and 28 cm^3 S+RSolid, 16 cm^3 S+RLiquid and, finally, control (S) 9 cm^3 at 150 days (Table 2). Rose *et al.* (1991) mention that root volume correlates positively with survival and growth, since these can prevail to transplanting stress, due to a greater capacity for water and nutrient hydration. Alzugaray *et al.* (2004)

reported that in plantations of the same species with greater volume, they presented a greater response to water stress by showing high concentrations of nutrients in the leaves, as well as a greater general growth of the plant.

The following researchers have shown that indole-3-butyric acid concentrations with a higher concentration (0.3%) provide the crop with better results in terms of root number, for example in *Dracaena deremensis* (Angulo, 2011). Rájala and Peltonen Sainio (2001) mention that applying growth regulators leads to an increase in root growth (increase in length and volume) and an increase in the root:stem ratio under field conditions. Likewise, Perez and Vertel (2010) found that macro, micro and trace elements in the rooting agent lead to this increase in plant growth.

In bird's-foot trefoil at 120 days it reached a length of 38 cm with Radix 1500 followed by Radix T 3000 with 34 cm and the control with 33 cm respectively.

The 0.15 (S+RSolid) and 0.3 % (S+RLiquid) AIB were applied as growth promoter and using soil + peat as substrates. Castrillón *et al.* (2008) report that AIB applications resulted in higher survival of cuttings in soil + peat substrate. Maldonado *et al.* (2017) in nanche *Malpighia mexicana* A., and *Byrsonima crassifolia* (L.) using Indolbutyric Acid (IBA) as rooting promoter in concentrations of 1000, 3000 and 10 000 ppm, and a control without rooting agent, planted in peat with sand, found that the two species of nanche presented low survival and sprouting, and concluded that the use of rooting promoters is necessary to obtain roots, and the propagation should be done under shade.

Leaf area (LA)

Leaf area began to increase from 30 days of age as the dry weight of leaves and the plant maturity increased; the maximum LA of 253 cm^2/g was presented at 150 days of age in S+RSolid, followed by S+RLiquid with 116 cm^2/g and control (S) with 46 cm^2/g, respectively. Bultynck *et al.* (1999) mention that LA is one of the main variables that can affect plant growth, by modifying the leaf area and the photosynthetic efficiency with respect to nitrogen use.

In the greenhouse where the experiment was carried out, the average temperature found was 8-43 °C (minimum and maximum, respectively). Ecke *et al.* (2004) found in studies carried out with Pascuas (*Euphorbia pulcherrima* Will. ex Klotzsch) that the ideal temperatures for rooting range between 22-24 °C if it propagates in a temperature higher than 26 °C, and its development is much slower and stops when the temperature is higher than 30 °C. Leakey and Mesén (1991) indicate experiences with other tropical species, where they show that the optimum air temperature that favors rooting is 20 to 25 °C; these data could have modified the results of the experiment in *Lotus corniculatus* L. since the plant height remained constant when temperatures were low.

CONCLUSIONS

The application of the rooting agent produced significant differences in the variables under study. The powdered rooting agent was the one that generated the most constant values, followed by the liquid and the control. The highest yields in green fodder and dry matter were achieved with the powdered rooting agent as the plant age increased from 30 to 150 days. Regarding the dynamics of root and aerial growth, the highest amount of DM accumulation occurred at four and five months. In addition, the use of rooting promoters is necessary to obtain a greater amount of roots, which help the plants to have an optimal development, reflected in the growth dynamics.

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