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Original Article

Effect of rhizobium and gibberellin on the production of hydroponic green forage of red clover (*Trifolium pratense* L.) variety quiñequeli

Uso de rizóbio e giberelina na produção de forragem verde hidropônica do trevo vermelho (*Trifolium pratense* L.), variedade quiñequeli

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Abstract

The purpose of the present investigation was to determine the effect of rhizobium and gibberellin on the production of hydroponic green forage from red clover (*Trifolium pratense* L.) variety quiñequeli, four variables were measured: plant height, biomass weight, grass weight and root weight. The treatments were T0: 0%, T1: 10%, T2: 20%, T3: 30% and T4: 40% of Rhizobium before germination and Gibberellin T0: 0g, T1: 2.4g; T2: 3.3g; T3: 4.3 and T4: 5.3g each treatment with 6 repetitions, three applications on days 5, 10, 15 and 20 of growth. Data were analyzed with DCA, ANOVA and DUNCAN's multiple comparisons test; the results obtained were: first measurement with rhizobium without gibberellin there were no statistical differences, second and third measurement with Gibberellin application did not present statistical differences and the fourth measurement presented statistical difference (α=0.05), average height of the plant with a mean of 12.82 cm, T4 was higher, in biomass a statistical difference was obtained with a mean of 3.056 kg, T3 was higher, weight of grass and root did not present statistical differences; concluding that the use of rhizobium and gibberellin could be a usable alternative in the production of hydroponic green fodder, to alleviate the problems of fodder scarcity in dry season, its use being recommended in high Andean livestock.

Keywords: soil less culture, biomass, yield, variety, silage.

Resumo

O objetivo da presente investigação foi determinar o efeito do rizóbio e da giberelina na produção de forragem verde hidropônica a partir do trevo vermelho (Trifolium pratense L.), variedade quiñequeli, para isso, foram medidas quatro variáveis: altura da planta, peso da biomassa, peso do capim e peso da raiz. Os tratamentos foram T0: 0%, T1: 10%, T2: 20%, T3: 30% e T4: 40% de Rizóbio antes da germinação, e Giberelina T0: 0g, T1: 2,4g; T2: 3,3g; T3: 4,3 e T4: 5,3g a cada tratamento com 6 repetições, com três aplicações nos dias 5, 10, 15 e 20 de crescimento. Os dados foram analisados com DCA, ANOVA e teste de comparações múltiplas de DUNCAN; os resultados obtidos foram: a primeira medição com rizóbio sem giberelina não apresentou diferenças estatísticas, a segunda e terceira medição com aplicação de giberelina não apresentaram diferenças estatísticas e a quarta medição apresentou diferença estatística (α=0,05), altura média da planta com média de 12,82 cm, T4 foi maior, na biomassa obteve-se diferença estatística com média de 3,056 kg, T3 foi maior, peso do capim e da raiz não apresentaram diferenças estatísticas; concluindo que o uso de rizóbio e giberelina poderia ser uma alternativa utilizável na produção de forragem verde hidropônica para aliviar os problemas de escassez de forragem na estação seca, sendo seu uso recomendado para a pecuária da região alta-andina.

Palavras-chave: cultura sem solo, biomassa, rendimento, variedade, silagem.

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1. Introduction

In the high Andean zone of Peru, during the year there are periods of drought, with characteristics of little or no rainfall and with very varied temperatures, which affects the development of crops, this phenomenon affects plants by limiting their growth and vegetative development; reason for which there is a shortage of natural and cultivated pastures, affecting the feeding of the animals, for this reason there is low production and productivity in the breeding of domestic camelids (Bediye et al., 2018).

In the Huancavelica region, as well as in all poor regions of Peru, agricultural activity is the basis of its economy, especially since the majority of the rural population is dedicated to this activity, and the production of alpacas represents one of its most large agricultural activities, but despite this, the majority of alpaca producers do not have technical support, making their mortality rate of these animals high (Neubert et al., 2021).

Currently, one of the most worrying problems in Huancavelica is the lack of food, adding to this; low land productivity, drastic environmental changes, peasant migrations, land abandonment, lack of opportunities and lack of water for irrigation. This has required the producer to think and devise technological methods to be able to meet the nutritional and nutritional needs of their cattle, which has led to the development of techniques such as the production of hydroponic green forage (Abdula, 2022).

There is limited scientific information in relation to the production of green forage of red clover (*Trifolium pratense* L.) variety quiñequeli using bacteria and phytohormones, so it is necessary to carry out various studies that provide information for this productive system.

Faced with this existing reality, the objective of the present investigation was to determine the effects of rhizobium and the plant hormone gibberellin on the production of hydroponic green forage of red clover variety quiñequeli as food in calves and pregnant mothers of alpacas in the dry season. Huancavelica region.

Regarding the review of literature and theoretical-scientific bases, the reports were obtained according to the study variables by different international and national researchers:

Red clover is an excellent forage plant of great importance for high Andean livestock as an alternative food in times of drought or dry seasons of the prairies, in which this biennial legume can substitute, with advantage, the cultivation of alfalfa (*Medicago sativa*) and other forage species with high protein content. Ranchers will easily be convinced of this and its cultivation will be generalized, as soon as they realize the few expenses it requires, the abundance of its products (50,000 and 60,000 kilograms of green fodder per hectare). All of this, together with the excellent quality of the product, which can be eaten green or teined by alpacas or other South American camelids, make it one of the most interesting fodder for our farmers who are dedicated to raising alpacas and llamas. greater than 4000 meters above sea level (Castañón, 2009).

This species of clover has deep roots, which can reach up to 1.5 meters, although they usually measure 0.5 to 0.6 meters. The stem reaches 0.40 to 0.080 meters, being generally hollow. The leaves consist of three oval or elliptical leaflets, and there is always a pair of opposite leaves, below and very close to the flower head (Castañón, 2009).

The production of hydroponic green fodder is considered a very effective way to supplement the diet of different domestic species, it is a technology for the production of nutrient-rich vegetable biomass in a total time of 12 days from its planting in the trays, which speeds up the availability of fresh fodder for cattle and allows its stabling or semi-stabling, due to its easy handling from the moment of planting to harvest on day 12, depending on the species that you want to use. Thus, constant forage availability and rapid renewal is of vital importance in times of forage scarcity, whether due to droughts, floods or simply because purchase prices are very high (Rosas, 2010).

The hydroponic green forage method is a practice totally isolated from the external environment due to its location inside a greenhouse, which allows the producer to control the environmental conditions for the production of forage of high nutritional and sanitary quality. Additionally, this method keeps the seed and forage out of the soil and away from all kinds of pests and diseases that can affect yield and quality, which does not happen in the case of sowing in pastures where the number of pests and weeds that delay or stop the growth of forage are innumerable (Estrada, 2002).

According to (Díaz, 2010) indicates that microorganisms capable of establishing symbiosis with legumes are known by the name of rhizobia or rhizobia and currently encompass a wide variety of bacteria. Knowledge of the existence of these microorganisms' dates from the end of the 19th century when, for the first time, GBIF Secretariat (2021) named the bacteria isolated from legume nodules Rhizobium legumin Sarum. It was this name that gave rise to the generic of rhizobia to designate all the nodule-forming bacteria in legumes.

Rhizobium bacteria are free-living organisms that inhabit the rhizosphere and feed on the remains of dead organisms. These contain a plasmid that encodes information that is vital for infection and nodulation of the corresponding host plant. They are motile, Gram-negative bacilli, with two layers of cell wall (the first layer is made of carbohydrates and proteins, and the second layer is made of lipids and carbohydrates), prokaryotes, aerobic (they need oxygen to grow), motile (on becoming motility test, the agar turns yellow and not its original purple color, beta (digests hemoglobin), grows almost at any temperature, but its development is most optimal at a temperature of 25°C (77°F), its dimensions are 0.5-0.9 x 1.2-3.0 µm, and it has flagella (Somasegaran and Hoben, 2012).

Also, phytohormones are organic compounds that are synthesized in one part of the plant and transferred to another part where, at very low concentrations, they exert a physiological response. Phytohormones are chemical substances, called hormones produced by some plant cells in strategic sites of the plant, the same ones that are predominantly capable of physiological phenomena and are produced in small quantities (Conversa et al., 2020). Gibberellin (GA_3) is a phytohormone that regulates various processes of plant development, which occur in the apical area, fruits and seeds. Its main functions are the

interruption of the latency period of the seeds, making them germinate, the induction of the development of buds, fruits and the regulation of the longitudinal growth of the stem, as well as the elongation of axial organs: petioles, peduncles, etc. (Hedden & Sponsel, 2015).

2. Materials and Methods

Peru is a South American country located in the region's northwest and situated between 70 and 81degrees west longitude and 0 and 18degrees south latitude. Also, Peru is located on west coast of South America, south of the Equator. Peru is home to over 27.94 million people, making it the seventh most populous nation in all of Latin America. The research was carried out in one of the greenhouses of the Pasture and Forage Experimental Center of the Professional School of Zootechnics of the National University of Huancavelica. At 3,680: m.a.s.l. with an average temperature of 17.3 °C, with an average relative humidity percentage at 68.04%, the experiment was carried out in January 2022. The greenhouse structure was made of wood with an area of 4 m^2, with a height of 1.80 m and a drop of 1.50 m, the roof was made of plastic, transparent tin roof with two waters. 30 plastic trays were used with an area of 1440cm ^{2} for each tray, with a sowing density of 300g per tray with a thickness of 0.3mm of red clover seed. A general overview of the experiment has been shown in Figure 1 and Figure 2.

The seeds were washed and disinfected with sodium hypochlorite, left to soak for 48 hours and the airing time was 12 hours during the night, followed by the application of the Rhizobium in different doses according to treatment with 10% sugar water. The dissolution was carried out in 167ml of water for each tray, after the installation of the trays in the modules, the seed was covered with absorbent paper to maintain humidity, for the dark stage or phase the greenhouse was completely covered with plastic black color, with a 75 ml non-chlorinated water supply with three irrigations during the first 4 days of germination, this was increased according to the need of the crop. Physiochemical properties of water used in the study are mentioned below in Table S1 (Supplementary Material).

For *Rhizobium* application, a strain of *phaseoli* were collected from Soil and Microbiological laboratory. The doses of Rhizobium per treatment were T0: 0%, T1: 10%, T2: 20%, T3: 30% and T4: 40% before germination and the dose of gibberellin applied at 5, 10, 15 and 20 days. For growth, plant height data was taken before and after the application of the hormone, 5 random measurements were taken from each tray. The harvest was carried out after 25 days, the variables evaluated were: average plant height, biomass weight, grass weight (stem + leaves) and root weight; A 50 cm long graduated ruler and a 3 kg capacity scale were used to collect the data. Which were analyzed in the ANOVA tables with DUNCAN multiple comparisons with a significance level α=0.05.

3. Results and Discussion

3.1. Plant height (cm) of red clover

The results for plant height (cm) of red clover are shown in Table 1.

The results obtained from Table 1, ANOVA of the measurements taken before the first application of gibberellin, shows that the treatments do not present statistical differences with Fc<Ft at a significance level of 0.05.

The results obtained from Table 2, ANOVA of the measurements taken before the second application of gibberellin, shows that the treatments do not present statistical differences with Fc<Ft at a significance level of 0.05.

The results obtained from Table 3, ANOVA of the measurements taken before the third application of gibberellin, shows that the treatments do not present statistical differences with Fc<Ft at a significance level of 0.05.

The results obtained from Table 4, ANOVA of the measurements taken on the day of harvest at 25 days, shows that the treatments present statistical differences with Fc>Ft at a significance level of 0.05. The DUNCAN test shows the differences between treatments in the following Table 5.

Figure 1. Distribución de bandejas a los 20 días después de la siembra.

Figure 2. Red clover (*Trifolium pratense* L.) variety quiñequeli 25 days old plants.

Table 1. ANOVA first measurement before the first application of gibberellin at 5 days of growth.

SV	DF	SC	CM	F calculated	F tabular	
					0.05	0.01
Treatment	4	0.89	0.22	1.54	2.76	4.18
Error	25	3.64	0.14			
Total	29	4.53				

DF = Degree of freedom; SC = Significant Characteristic; CM = Sum of the data values divided by the number of values; F = represent to values obtained from statistical software; SV = represents the elements in column.

Table 2. ANOVA second measurement before the second application of gibberellin at 10 days of growth.

SV	DF	SC	CM	F calculated	F tabulated	
					0.05	0.01
Treatment	4	2.84	0.71	0.85	2.76	4.18
Error	25	20.75	0.83			
Total	29	23.60				

DF = Degree of freedom; SC = Significant Characteristic; CM = Sum of the data values divided by the number of values; F = represent to values obtained from statistical software; SV = represents the elements in column.

Table 3. ANOVA third measurement before the third application of gibberellin at 15 days of growth.

SV	DF	SC	CM	F calculated	F tabulated	
					0.05	0.01
Treatment	4	2.61	0.65	0.92	2.76	4.18
Error	25	17.61	0.70			
Total	29	20.23				

DF = Degree of freedom; SC = Significant Characteristic; CM = Sum of the data values divided by the number of values; F = represent to values obtained from statistical software; SV = represents the elements in column.

Table 4. ANOVA fourth measurement before the fourth application of gibberellin at 20 days of growth.

SV	DF	SC	CM	F calculated	F tabulated	
					0.05	0.01
Treatment	4	13.64	3.41	5.17	2.76	4.18
Error	25	16.46	0.65			
Total	29	30.11				

DF = Degree of freedom; SC = Significant Characteristic; CM = Sum of the data values divided by the number of values; F = represent to values obtained from statistical software; SV = represents the elements in column.

The DUNCAN test shows that there are statistical differences, determining that the average height in T0 is lower than T1, T2, T3 and T4.

It can be explained in terms of the existence of microorganisms, such as bacteria of the Rhizobium genus, which fix nitrogen symbiotically with leguminous plants. The use of commercial inoculants based on Rhizobium is a widespread practice for farmers, in order to increase yields with low costs (Calero et al., 2019).

The results are corroborated by Rodríguez (2020), Rhizobium inoculation promotes the growth of the aerial part of the plant, showing an increase in stem length of up to approximately 50%.

Likewise, gibberellins are growth regulators that will transmit to cells the necessary messages to influence the development and differentiation of tissues. Although no specific receptors have been found, it is known that differential concentrations of this regulator cause different

Table 5. The numbers obtained from the Duncan test data collection are provided

		Difference		ALS (D)	significance
SAW	12.8-10.8	2.0	>	0.311	*
V-II	12.8-11.4	1.4	>	0.305	*
V-III	12.8-11.9	0.9	>	0.297	*
V-IV	12.8-12.2	0.6	>	0.282	*
IV-I	12.2-10.8	1.4	>	0.305	*
IV-II	12.2-11.4	0.8	>	0.297	*
IV-III	12.2-11.9	0.3	>	0.282	*
III-I	11.1-10.8	0.8	>	0.297	*
III-II	11.9-11.4	0.5	>	0.282	*
II-I	11.4-10.8	0.6	>	0.282	*
T0	T1	T2	T3	T4	
10.8	11.4	11.9	12.2	12.8	
And	d	c	b	a	

SAW = represents the control; ALS = represents the standard deviation. Letters are representing the significant difference among all treatments. T0 = 0% rhizobium + 0g gibberellins; T1 = 10% rhizobium + 2.4g gibberellins; T2 = 20% rhizobium + 3.3g gibberellins; T3 = 30% rhizobium + 4.3g gibberellins; T4 = 40% rhizobium + 5.3g gibberellins. Here * represents the significance of the results.

Table 6. ANOVA of the weight (kg) of the biomass.

SV	DF	SC	CM	F calculated	F tabulated	
					0.05	0.01
Treatment	4	0.717	0.179	3.82	2.76	4.18
Error	25	1,173	0.046			
Total	25	1,173				

DF = Degree of freedom; SC = Significant Characteristic; CM = Sum of the data values divided by the number of values; F = represent to values obtained from statistical software; SV = represents the elements in column.

physiological effects in the plant, such as cell elongation (Mishra et al., 2022).

An alternative is growth hormone (Gibberellins) that is currently used in applications to legume crops to improve yield (Rafique et al., 2021).

In general, the plant heights recorded in the trial were comparatively the same as described by FAO (2001), which suggested that red clover (*Trifolium pratense* L.) variety quiñequeli is a succulent food of approximately 20-30 cm. under greenhouse conditions (18-26 °C and 90% RH) and with chemical solutions of macro and micro nutrients.

3.2. Biomass weight (kg) of red clover

The results for biomass weight (kg) of red clover are shown in Table 6.

The results show us that the biomass weight in the different treatments applied is statistically different with Fc>Ft at a significance level of 0.05; for which the DUNCAN test was performed, which is shown in the following Table 7.

The results found indicate that, in the first evaluation cycle, red clover concentrates between 66 - 70% of the production for the different cultivars (López-Olivari and Ortega-Klose, 2021).

What differs with Kenana, (2022) in their research since they found higher values of fresh biomass with 3.54 kg/m ^{2} with minimal differences compared to ours.

To obtain a positive response to Rhizobium bacterial inoculation on plant development and yield, it is necessary for the bacterial population to reach a significant biomass (Aguirre et al., 1999).

As stated by Ruiz et al. (2006), that biomass production is a multidisciplinary activity; Hence, the success of its operation is conditioned to the knowledge of the interactions between its components, as well as between these and the environment, which will allow the generation of management strategies in accordance with the ecology that lead to improve productivity and sustainability. of the agroecosystem. Therefore, biomass production is a determining element in the success and efficiency of systems with legumes.

Under other conditions, there is greater production of biomass as Korner (2013) affirms that solar radiation, water supply and nutrients are the most important

physical -chemical drivers in plant life, determining the assimilation of carbon dioxide and production. of biomass.

3.3. Grass weight (kg) of red clover

The results for grass weight (stems + leaves) of red clover are shown in Table 8.

The quiñequeli variety red clover has a high leaf/stem ratio that ensures high quality forage. It is a material that adapts to direct grazing in the same way as cutting or tedding (Picasso, 2011).

The beneficial effect of inoculation with Rhizobium in legumes was reported by González et al. (2012), who increased the number of trifoliate leaves in relation to the control without inoculation. In this sense, Marin et al. (2013), with the association between Tsukamurella paurometabola C-924 and Rhizobium leguminosarum in legumes, managed to significantly stimulate the number of leaves in relation to the control without inoculation.

These results agree with what was mentioned by Eriksson et al. (2000) who indicate that the application of GAs increases the size of the meristematic zone by increasing the number of cells that enter into cell division and these subsequently contribute to stem elongation, leaf growth. and roots; which translates into an increase in the fresh weight of the seedling.

The application of gibberellins to the stems produces a pronounced increase in cell division in the subapical meristem (Sach et al., 1960 cited by Weaver, 1996). These results also establish that GAs directly influence the growth and development of seedlings.

3.4. Root weight (kg) of red clover

The results for weight (kg) of the red clover root are shown in Table 9.

The root system is initially formed by the radicle, which later becomes the main or primary root. The main root is easily identifiable by its diameter and its position below the stem. Subsequently, the secondary roots emerge, especially in the upper part of the main root, arranged in the shape of a crown. Tertiary roots form laterally on secondaries and quaternary roots on tertials. Roots of a higher order than those mentioned can be observed (Karavidas et al., 2022).

Table 7. DUNCAN test weight (kg) of biomass.

		Difference		ALS (D)	significance
SAW	2,737-2,581	0.156	<	0.311	DK
V-II	2,737-2,796	-0.059	<	0.305	DK
V-III	2,737-2,737	0	<	0.297	DK
V-IV	2,737-3,056	-0.319	<	0.282	DK
IV-I	3,056-2,581	0.475	>	0.305	*
IV-II	3,056-2,796	0.260	<	0.297	DK
IV-III	3,056-2,737	0.319	>	0.282	*
III-I	2,737-2,581	0.26	<	0.297	DK
III-II	2,737-2,796	-0.059	<	0.282	DK
II-I	2,796-2,581	0.215	<	0.282	DK
T0	T1	T2	T3	T4	
2.58	2.79	2.73	3.06	2.73	
C	b	b	a	b	

To the DUNCAN test, it shows that T3 compared to T0, T1, T2 and T4 is superior with a significance level of 0.05. SAW = represents the control; ALS = represents the standard deviation. Letters are representing the significant difference among all treatments, T0 = 0% rhizobium + 0g gibberellins; T1 = 10% rhizobium + 2.4g gibberellins; T2 = 20% rhizobium + 3.3g gibberellins; T3 = 30% rhizobium + 4.3g gibberellins; T4 = 40% rhizobium + 5.3g gibberellins. Here * represents the significance of the results.

Table 8. ANOVA of the weight (kg) of the grass.

SV	DF	SC	CM	F calculated	F tabulated	
					0.05	0.01
Treatment	4	0.112	0.028	0.829	2.76	4.18
Error	25	0.847	0.033			
Total	29	0.959				

It is shown that there are no statistical differences with an Fc<Ft at a significance level of 0.05. DF = Degree of freedom; SC = Significant Characteristic; CM = Sum of the data values divided by the number of values; F = represent to values obtained from statistical software; SV = represents the elements in column.

Table 9. ANDEVA of the weight (kg) of the root.

SV	DF	SC	CM	F calculated	F tabulated	
					0.05	0.01
Treatment	4	0.076	0.019	0.308	2.76	4.18
Error	25	1,548	0.061			
Total	29	1,624				

The analysis in the ANOVA table shows that the treatments had no statistical differences with Fc<Ft at a significance level of 0.05. DF = Degree of freedom; SC = Significant Characteristic; CM = Sum of the data values divided by the number of values; F = represent to values obtained from statistical software; SV = represents the elements in column.

In reference to the dose of rhizobium inoculum, Bidwell (1979) says that a high inoculation achieves success in nitrogen fixation, because the bacterium is specific for this legume, also Liu et al. (2020), mentions that the roots Nitrogen-deficient plants tend to have few branches.

The bacteria stimulate the growth of stems and roots, which is consistent with Perrine et al. (2004), who state that Rhizobium sp. bacteria produce high concentrations of indole acetic acid (IAA), which stimulates seed growth.

The results differ from those obtained by Mayak et al. (2004) who state that Rhizobium can produce ACC (amino cyclopropane carboxylate) deaminase; compound that reduces the level of ethylene in the roots of plants, thus increasing the length and growth of the roots.

4. Conclusions

Rhizobium and Gibberellin present a positive effect on plant growth and development, that is, it presented a statistical difference for the average plant height variable where T0 is lower than T1, T2, T3 and T4. In the same way, for the biomass variable, I also present a statistical difference. The test shows us that T3 compared to T0, T1, T2 and T4 is higher with a significance level of 0.05. However, for grass weight and root weight there was no statistical difference with Fc<Ft at a significance level of 0.05.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Physiochemical properties of water used in the study.

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