INFLUENCE OF *AZOLLA-ANABAENA* SYMBIOSIS ON RICE (*Oryza sativa* L) CROP AS A NUTRITIONAL ALTERNATIVE

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ABSTRACT. With the objective of studying the influence of *Azolla* as green manure or associated to rice crop under controlled conditions, an experiment was carried out during the period of May-October, 1997, using a randomized complete design. 16 treatments were evaluated resulting from the combination of four nitrogen levels (0, 40, 80, 120 kg.ha^{-1} N) and four cropping systems (rice as monoculture, rice+incorporated *Azolla*, rice+incorporated *Azolla* + associated *Azolla*, rice+associated *Azolla*) and each treatment was replicated four times. Results showed that rice was positively influenced by the use of *Azolla* in all variants used, emphasizing the combination of fern incorporation and its association with rice. This allowed to increase plant height, number of effective tillers, leaf area and dry matter, as well as a noticeable increment in nutrient extraction by this cereal. The use of *Azolla* also increased organic matter and potassium contents of the soil. A positive effect was also observed by increasing nitrogen fertilization levels.

Key words: rice, *Anabaena*, *Azolla*, symbiosis, alternative agriculture, plant nutrition

RESUMEN. Con el objetivo de estudiar la influencia de la asociación o incorporación de *Azolla* en el cultivo del arroz en condiciones controladas, se llevó a cabo un experimento durante el período comprendido entre mayo y octubre de 1997, usando un diseño completamente aleatorizado. Se evaluaron 16 tratamientos, productos de la combinación de cuatro niveles de nitrógeno (0, 40, 80, 120 kg N.ha^{-1}) y cuatro sistemas de cultivo (arroz en monocultivo, arroz+*Azolla* incorporada, arroz+*Azolla* incorporada+*Azolla* asociada, arroz+*Azolla* asociada) y cada tratamiento fue replicado cuatro veces. Los resultados mostraron que el arroz fue influido de forma positiva por el uso de *Azolla* en todas las variantes usadas, destacándose la combinación de incorporación del helecho y la asociación de este con el cultivo del arroz, lo que permitió incrementar la altura de las plantas, el número de hijos fértiles, el área foliar y su masa seca, así como provocó un aumento notable en la extracción de nutrientes por este cereal. El uso de *Azolla* incrementó los contenidos de materia orgánica y potasio del suelo. Se observó también un efecto positivo al incrementar la dosis de fertilización nitrogenada.

Palabras clave: arroz, *Anabaena*, *Azolla*, simbiosis, agricultura alternativa, nutrición de las plantas

INTRODUCTION

The use of biofertilizers and green manures is not only an agribusiness need in underdeveloped countries, but also an ecologically balanced and economically feasible scientific agriculture (1).

Plants used as green manure are generally able to get atmospheric nitrogen, by means of symbiotic or nonsymbiotic associations with nitrogen-fixing bacteria or cyanobacteria, such as leguminous, *poaceas*, and some fern species (1).

In Asia, *Azolla* is the most used green manure on rice crop, due to its high capacity for multiplying and fixing nitrogen, it being able of providing rice plantations with 600 kg N per hectare per year (2, 3).

The numerous advantages of using such fern are not well known in our country. According to bibliography, *Azolla* can supply more than half the required nitrogen to rice crop. It also reduces water and nitrogen losses, as well as mosquito proliferation, regulates pH and water temperature, and increases crop yield (4).

Azolla caroliniana is considered an Azolla species of Cuban flora, which is found in places where climatic conditions are favorable for fern to grow. However, poor attention has been put to its use, since there is lack of knowledge and culture for using green manures on rice crop.

Therefore, the present study was carried out, based on the hypothesis that using *Azolla* could constitute a feasible nutritional alternative to be applied under Cuban conditions, since the amounts of nitrogen it provides to the soil or its intercropping in paddy fields, could favor rice growth and development and increase yield. Consequently, the main objective of this work was to study the influence of *Azolla* as green manure or associated crop on rice development.

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MATERIALS AND METHODS

The work was conducted at the areas from "Los Palacios" Rice Research Station, belonging to the National Institute of Agricultural Sciences and located in "Los Palacios" Rice Agroindustrial Complex, Pinar del Río.

A hydromorphic gley nodular ferruginous soil was used (5), presenting the following chemical properties (Table I).

Table I. Initial soil chemical features at the areas from "Los Palacios" Rice Research Station

Indexes	Values	Methods
Organic matter OM (%)	3.29	Walkley-Black
Phosphorus P (ppm)	63.0	Oniani (extraction with H_2SO_4 0.1N)
Potassium K (meq.kg^{-1})	0.21	Oniani (extraction with H_2SO_4 0.1N)
Calcium Ca (meq.kg^{-1})	18.3	Maslova ($CH_3COO\ NH_2$), pH7, 1N
Magnessium Mg (meq.kg^{-1})	2.7	Maslova ($CH_3COO\ NH_2$), pH7, 1N
PH (H_2O)	6.2	Potentiometric

Soil chemical features were adequate for developing the experiment, whenever the recommended fertilization was applied.

Water used for irrigation presented appropriate contents of total salts and electric conductivity.

Climatic variables, recorded at Paso Real de San Diego Meteorological Station (Table II), presented a steady behavior with no significant differences during the evaluated period in relation to historical means.

Table II. Mean monthly temperature, mean monthly rainfall, and mean monthly relative humidity (1997)

Variable	1997					
	M	J	J	A	S	O
Mean monthly temperature (ºC)	27.1	26.8	27.6	27.7	26.4	25.6
Mean monthly rainfall (mm)	45.2	314.7	105.2	107.2	279.2	38.4
Mean monthly relative humidity	79	86	82	83	89	84

Three *Azolla* ecotypes were used, which are presented in Table III.

Table III. *Azolla* ecotypes used

Ecotypes	Species	Origin	Color
UPGL-10	*Azolla filiculoide* (hybrid)	Belgium	Unpolished green
MI-69	*Azolla filiculoide* (hybrid)	Belgium	Green/Pink
APR-1	*Azolla caroliniana*	Cuba (P. del Río)	Bright green

Urea, triple super phosphate and potassium chloride were the NPK carriers used, following the recommended dose for rice crop (6, 7).

The experiment was developed in 0.018 m^3 pots, presenting an area of 0.06m^2, during the period of May-October, 1997. Nine plants from rice J-104 variety were put per pot.

Azolla was incorporated to the soil as green manure, at the rate of 0.12 kg.pot^{-1} (20 t.ha^{-1}), and mixed with it before seeding, when it was required. Initial inoculum application was a dose of 0.018 kg per pot (3 t.ha^{-1}), after establishing water table.

Equal shares of ecotypes APR-1, UPLG-10 and MI-69 were mixed since, in previous studies, this method proved to be more effective for achieving a more stable plant growth and pest tolerance, than using such ecotypes separately.

A randomized complete design with four repetitions and 16 treatments was used as follows:

1. O N (Control)
2. 1/3 N
3. 2/3 N
4. 3/3 N (Check)
5. O N + incorporated *Azolla*
6. 1/3 N + incorporated *Azolla*
7. 2/3 N + incorporated *Azolla*
8. 3/3 N + incorporated *Azolla*
9. O N+ incorporated *Azolla*+topdressing *Azolla*
10. 1/3 N + incorporated *Azolla*+topdressing *Azolla*
11. 2/3 N + incorporated *Azolla*+topdressing *Azolla*
12. 3/3 N + incorporated *Azolla*+ topdressing *Azolla*
13. O N + topdressing *Azolla*
14. 1/3 N + topdressing *Azolla*
15. 2/3 N + topdressing *Azolla*
16. 3/3 N + topdressing *Azolla*

Nitrogen applications were splitted according to the Ministry of Agriculture and doses of the studied treatments were divided by variant (6).

Doses applied to each treatment:

O N - (Control)
1/3 N - 0.24 g N per pot (40 kg.ha^{-1})
2/3 N - 0.48 g N per pot (80 kg.ha^{-1})
3/3 N - 0.72 g N per pot (120 kg.ha^{-1})

Measurements performed:

- Plant height or the topmost plant tip (at 100 % heading)
- Number of fertile tillers per plant (at 100 % heading)
- Dry mass of plant top (at 100 % heading)
- Leaf analysis (N, P, K) at 50 %heading
- Soil chemical analysis (OM, pH, P, K, Ca and Mg). Sampling was performed 45 days after incorporating rice plants and *Azolla*.

RESULTS AND DISCUSSION

Figure 1 shows the behavior of rice plant height, influenced by *Azolla* incorporation and/or association as well as different N rates. An increase for this character is seen in treatments where incorporated and/or associated *Azolla* was used. However, nitrogen dose and *Azolla* influence did not show significant differences, except for treatments in which 1/3 doses N with T6 incorporated *Azolla* and 2/3 doses N with T11 incorporated and associated *Azolla* were applied.

Treatments from T7 to T12 and T14 to T16, in which incorporated and/or associated *Azolla* was used, presented the greatest plant height values, without showing significant differences among them. The highest value was achieved by treatment T11, where 80 kg.ha^{-1} N was applied to rice, together with the incorporation and association of *Azolla* in this crop.

Treatment T9 stood out, through which nitrogen was not applied and both ways of using *Azolla* were performed. This treatment seems to supply nitrogen demands of rice plants.

It was also observed that treatments where *Azolla* was used without nitrogen also showed better behavior with significant differences in relation to the control, which presented a poor plant growth, just 67.25 cm high.

In general terms, such behavior of rice height was caused by the influence of nitrogen content in the medium, which could be supplied through a different way and nature, as it is proved in recent publications (2, 8).

Figure 2 shows the number of effective tillers per plant. Nitrogen doses applied together with *Azolla* in this experiment influenced this variable as well. Treatments where *Azolla* was incorporated and/or associated (T8, T12, T7, T11 and T16) stood out, for presenting the highest number of tillers per plant, without showing significant differences among them.

Figure 3 shows nitrogen extracted by rice plants and plant dry mass at different variants, up to 100 % flowering.

Like the number of tillers per plant and height, nitrogenous fertilization along with *Azolla* influenced nitrogen extracted by plants and dry mass production.

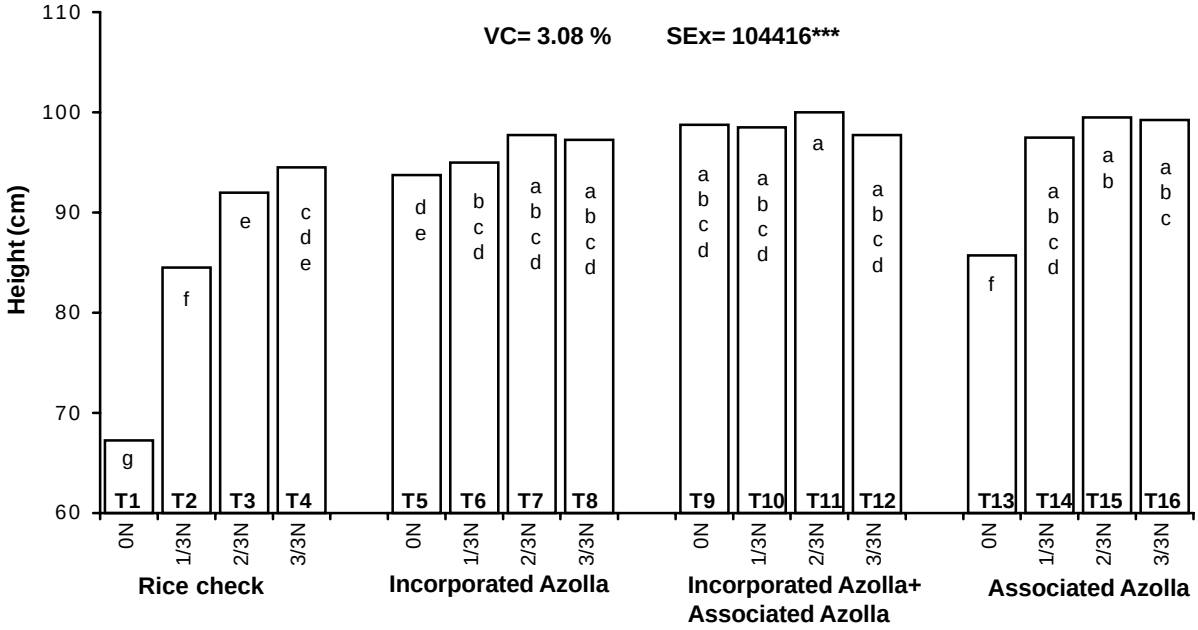

Figure 1. Influence of treatments on rice plant height

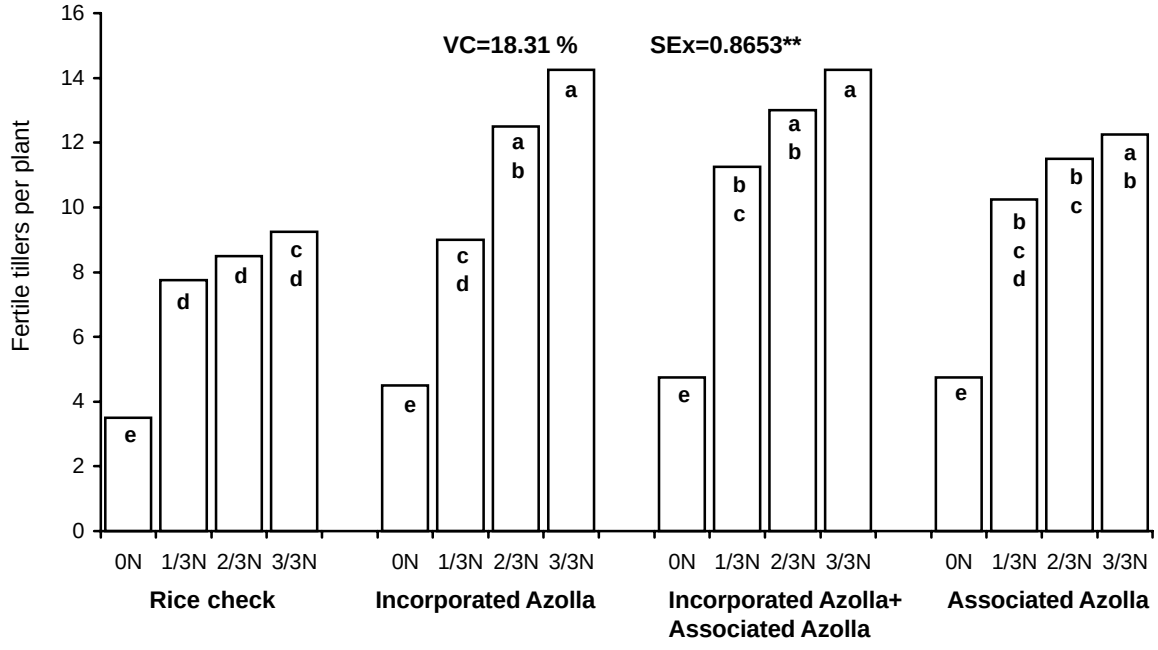

Figure 2. Influence of treatments on the number of fertile tillers

Treatments combining both ways of using *Azolla* tended to produce more biomass than the remaining treatments with the same nitrogen dose. Therefore, combining incorporation and association of such fern not only serves to provide plants with significant amounts of nitrogen, but also enables a better use of nitrogen added by mineral fertilization, and, thereby, a higher dry mass production. Similar results were recorded when using *Azolla pinnata* (8, 9).

For both variables, it is highlighted the fact that nitrogen, phosphorous and potassium doses are inadequate for achieving good crop development; however, the influence of incorporated and/or associated *Azolla* allows a better use of nitrogen and better conditions for assimilating other nutrients, thus improving crop nutritional stage.

Treatments T8 and T12 presented the highest nitrogen concentrations in plants, without significant differences. In both of them, incorporated *Azolla* and 120 kg.ha^{-1} N were used. The incorporation of this fern seems to maintain a higher soil nitrogen availability than its association, which coincides with other results (10, 11, 12).

Regarding dry mass production and nitrogen content, in treatments where incorporated and associated *Azolla* were combined, values tended to be higher, compared to other treatments presenting the same nitrogen doses. This is caused by the influence of incorporated and associated *Azolla* on plant available nitrogen content in the soil and water, as a result of nitrogen release during fern decomposition, reduction in the loss of nitrogen applied as fertilizer and the one excreted to water by associated *Azolla* (4).

Figure 4 shows the influence of associated and/or incorporated *Azolla* on phosphorus and potassium contents in rice plants, with different nitrogen doses, where both nutrients presented similar behavior to that of nitrogen, positively influenced by N fertilization and *Azolla*. In general, combining both ways of using *Azolla* surpassed the remaining treatments, followed by variants where *Azolla* was associated to rice crop, and where fern was incorporated. This could be owing to the influence of supplying these elements when fern is decomposed, as well as to the effect on pH provoked by associated *Azolla*, which increase solubility of such elements, according to other results presented in Japan (13). It was also proved that *Azolla* increased fertilizer efficiency, mainly when applying incorporated and associated *Azolla*.

When analyzing soil chemical features after incorporating rice straw and *Azolla* (Table IV), it could be noticed that treatments did not influence pH, P, Ca nor Mg. However, they did influence organic matter and potassium contents.

Different from the remaining variables, OM and K contents in the soil presented significant differences among treatments; the use of *Azolla* and dry matter incorporation influenced both of them. In this sense, the highest values were achieved by combining incorporated and associated *Azolla*, followed by treatments where *Azolla* was incorporated, leaving the third place to treatments in which such fern was associated to rice crop. This is related to the contribution of *Azolla* when decomposed and rice dry matter, even though the latter had not got its full decomposition.

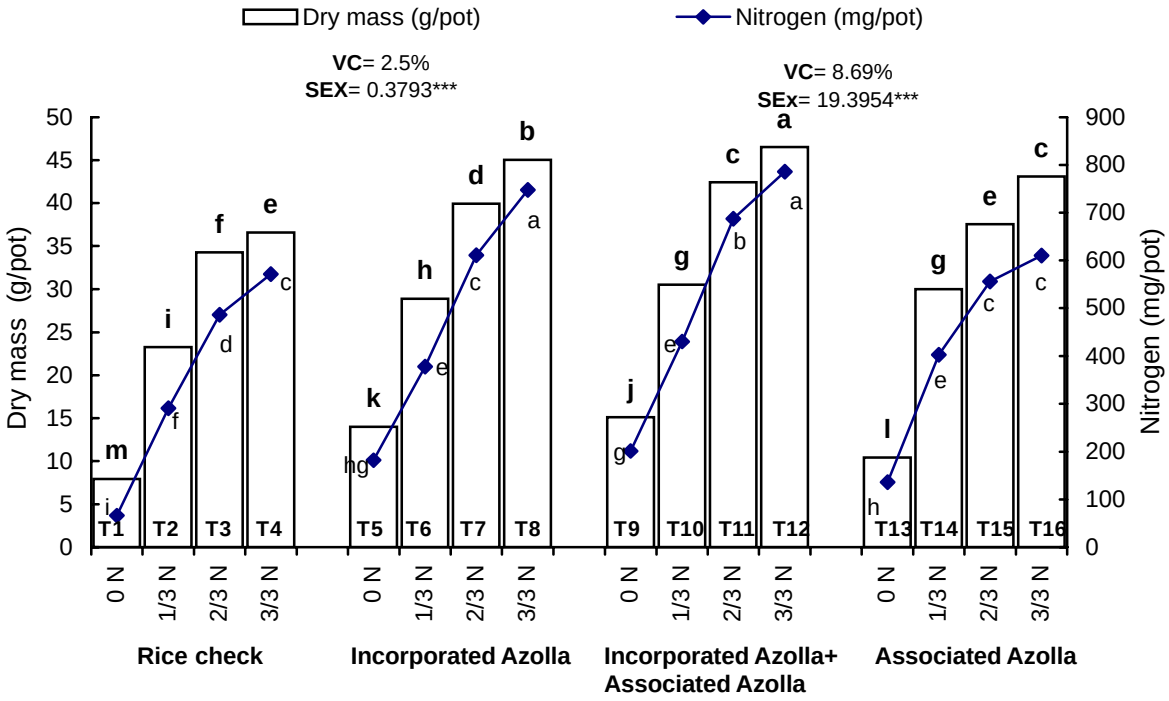

Figure 3. Influence of treatments on dry mass and nitrogen content

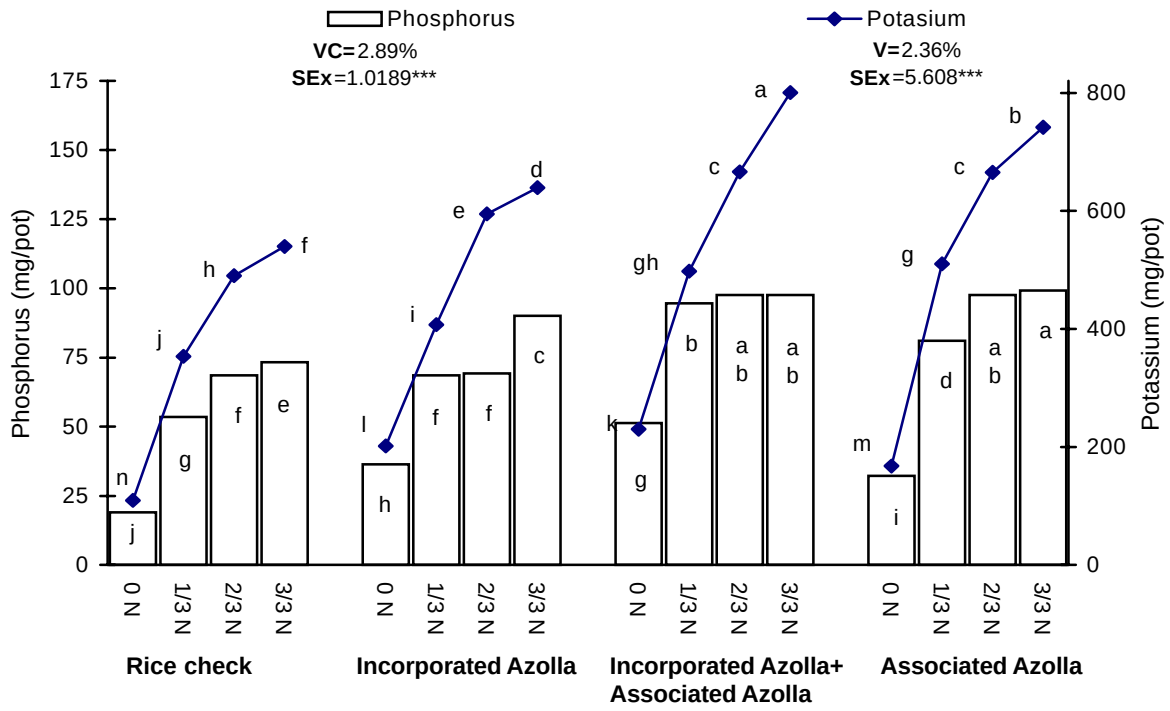

Figure 4. Influence of associated and/or incorporated *Azolla* on phosphorus and potassium contents in rice plants with different nitrogen doses

Table IV. Soil features after incorporating harvest remains

Treatments		pH	OM	P	K	Ca	Mg
ON	T1	6.72	3.92 ef	65.00	0.23 e	19.37	3.22
1/3N	T2	6.75	3.90 f	63.75	0.25 de	19.50	3.75
2/3N	T3	6.75	3.79 f	60.75	0.24 de	19.37	3.50
3/3N	T4	6.75	3.68 f	66.25	0.26 cde	19.25	3.75
ON	T5	6.80	4.45 bcde	62.50	0.34 abc	19.37	3.00
1/3N	T6	6.75	4.51 bcde	61.00	0.32 bcd	19.50	3.00
2/3N	T7	6.77	4.51 bcde	61.25	0.3 bcde	19.37	3.12
3/3N	T8	6.77	4.88 ab	67.50	0.35 ab	19.50	3.00
ON	T9	6.75	4.73 bc	62.50	0.35 a	20.25	3.00
1/3N	T10	6.80	4.72 bc	68.25	0.32 abcd	19.50	3.37
2/3N	T11	6.82	4.88 ab	67.00	0.31 abcd	19.50	3.25
3/3N	T12	6.77	4.99 ab	63.75	0.34 abc	19.75	3.37
ON	T13	6.82	4.13 dfe	65.00	0.28 bcde	19.50	3.12
1/3N	T14	6.80	4.16 cdef	62.50	0.20 bcde	19.75	3.25
2/3N	T15	6.82	4.22 cd	64.50	0.30 bcde	19.37	3.62
3/3N	T16	6.80	4.20 cdef	68.75	0.31 bcde	19.87	3.25
VC		0.96 %	8.05 %	10 %	16.06 %	4.65 %	17.98 %
SE x		0.33 ns	0. 1759*	3.2196 ns	0.243***	0.4543 ns	0.2934 ns

Researchers in Asia, using transplantation technology as well as *japonica* and *indica* varieties recorded similar results (8).

In general terms, *Azolla* benefits rice crop, obtaining the highest response when combining incorporation and association of such fern. It also favors nutrient absorption by this cereal and increases its yield, as well as organic matter content of the soil.

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