Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713737104

Culture of *Azolla filiculoides* in artificial conditions

A. L. Pereira [a]; F. Carrapiço [a]
[a] Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Vegetal, Centro de Biologia Ambiental, 1749-016 Lisboa, Portugal

First published on: 24 November 2009

To cite this Article Pereira, A. L. and Carrapiço, F.(2009) 'Culture of *Azolla filiculoides* in artificial conditions', Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology,, First published on: 24 November 2009 (iFirst)
To link to this Article: DOI: 10.1080/11263500903172110
URL: http://dx.doi.org/10.1080/11263500903172110

Plant Biosystems, 2009, 1–4, iFirst Article

Taylor & Francis
Taylor & Francis Group

Culture of *Azolla filiculoides* in artificial conditions

A. L. PEREIRA & F. CARRAPIÇO

Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Vegetal, Centro de Biologia Ambiental, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal

Abstract
Azolla filiculoides showed a planar development in four culture media, but with overlapping of sporophytes after 28 days, and curled roots in all cases except for IRRI2. The difference in biomass between the media IRRI2 and IRRI1-Fe10x was statistically significant at Days 14, 21 and 28 by ANOVA. Medium IRRI2 gave the highest duplication time.

Keywords: Azolla filiculoides, *biomass, culture, duplication time, relative growth rate*

Introduction

Azolla Lamarck is a small floating aquatic pteridophyte with bilobed leaves (dorsal and ventral lobes). The dorsal lobe has an ovoid cavity in which inhabits the nitrogen-fixing cyanobacterium *Anabaena azollae* Strasburger. This unique association is important as rice culture biofertiliser, animal feed or wastewater purifier (Carrapiço et al. 2000).

The nutrients (macro- and micronutrients) and abiotic factors (light, temperature and pH) in an artificial culture are important for an excellent plant growth and development (Pierik 1987). In the culture of *Azolla*, which resembles a hydroponic culture, the most frequent media are Hoagland (Allen & Arnon 1955; Peters et al. 1980) and IRRI media (Watanabe et al. 1977). Optimised growth systems in controlled conditions are important for the production of large amounts of biomass and for ensuring the good quality of *Azolla* if the goal is to provide this pteridophyte as animal feed.

The aim of the present study was to compare the relative growth rate (RGR), duplication time (DT) and production of biomass of *Azolla filiculoides* growing in four different culture media (H-40, IRRI1, IRRI1-Fe 10x and IRRI2) in artificial conditions.

Materials and methods

Growth of Azolla filiculoides *in culture media*

Azolla filiculoides were placed in uncovered round PVC boxes (11 × 18 cm) in a culture chamber. The fern was kept at a temperature of 23–24°C, photoperiod of 16 h light/8 h dark, relative humidity of 55.8% and light intensity of 6.0 Wm^{-2} (Phillips Cool White TLD 36W). The tested media were Hoagland (H-40) (Allen & Arnon 1955; Peters et al. 1980), IRRI1-Fe1x (Watanabe et al. 1977) and IRRI2 (T. Ventura, personal communication, 10 May 2002) (Table I). The IRRI1-Fe10x medium has an iron concentration 10 times higher than that of IRRI1-Fe1x medium. The medium was renewed every 28 days.

Relative growth rate and duplication time

Four replicas of acclimatised *A. filiculoides* were used to estimate the RGR and DT. The biomass inoculated in each PVC box was approximately 4 g and the medium was not replaced throughout the experiments. Weekly (T = 7, 14, 21 and 28 days) the fern was removed from the PVC boxes, placed on absorbent paper to remove the excess of liquid and weighted. The RGR and DT were assessed by standard formulae (Pabby et al. 2001).

Correspondence: A. L. Pereira, Centro de Biologia Ambiental, Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Rua Ernesto Vasconcelos, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal. Tel: +351 21 750 03 81. Fax: +351 21 750 00 48. Email: anapereira271268@yahoo.com

ISSN 1126-3504 print/ISSN 1724-5575 online
DOI: 10.1080/11263500903172110

Table I. Macronutrients, micronutrients and iron source of the culture media used for the growth of *A. filiculoides*.

	H-40	IRRI1-Fe1x	IRRI2
Macronutrients			
$CaCl_2$. $2H_2O$	1.50 mM	0.7 mM	40 μM
$MgSO_4.7H_2O$	0.80 mM	1.6 mM	40 μM
NaCl	0.50 mM	–	–
KH_2PO_4	0.40 mM	–	–
$K_2HPO_4.3H_2O$	0.05 mM	–	–
KCl	2.00 mM	–	–
NaH_2PO_4	–	0.7 mM	–
K_2SO_4	–	0.5 mM	40 μM
$NaH_2PO_4.H_2O$	–	–	20 μM
Micronutrients			
$CuSO_4.5H_2O$	0.3 μM	3.2 μM	0.01 μM
$MnSO_4.H_2O$	9.1 μM	27.2 μM	–
H_3BO_3	46.3 μM	3.2 μM	0.20 μM
$Na_2MoO_4.2H_2O$	1.0 μM	0.2 μM	0.15 μM
$ZnSO_4.7H_2O$	0.8 μM	–	0.01 μM
$CoCl_2.6H_2O$	0.2 μM	0.2 μM	0.01 μM
$MnCl_2.4H_2O$	–	–	0.50 μM
Iron source			
$Fe\text{-}EDTANa_2$	7.2 μM	–	0.50 μM
$FeC_6H_5O_7$	–	35.5 μM	

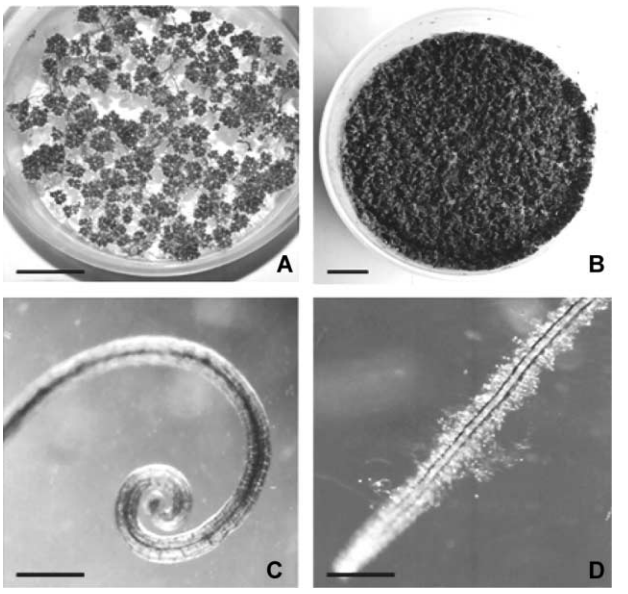

Figure 1. Sporophyte and roots of *A. filiculoides* after 28 days of growth in artificial media. (A) Planar growth in medium H-40; (B) overgrowth in medium IRRI2; (C) curled root in medium IRRI1-Fe1x; (D) uncurled root in medium IRRI2. Scale bar = 5 cm (A–B); 200 μm (C–D).

Statistical analysis

One-way ANOVA (*F*-test) and Tukey (*q*-test) with $\alpha = 0.05$ (Zar 1999) were applied to the biomass at 7, 14, 21 and 28 days for each medium. The differences are statistically significant if $F > F_{0.05,3,12} = 3.49$ for ANOVA and $q > q_{0.05,12,4} = 4.199$ for Tukey.

Results

The sporophyte of *A. filiculoides* in all the culture media tested showed a planar growth (Figure 1A), but as the biomass increased overlapping of sporophytes was observed (Figure 1B) without necrosis. The roots curled at the tip (Figure 1C), although on IRRI2 medium root curling was not evident (Figure 1D).

The RGR were similar on all the media, varying from 0.03 g day^{-1} for H-40, IRRI1-Fe1x and IRRI1-Fe10x to 0.04 g day^{-1} for IRRI2. The DT was shorter for IRRI2 (8.43 days), longer for IRRI1-Fe10x (11.91 days) and intermediate for IRRI1-Fe1x (8.99 days) and H-40 (10.36 days).

The biomass of *A. filiculoides* was analysed by ANOVA (Figure 2). On Days 14, 21 and 28 there was a significant increase in the biomass, mainly for IRRI1-Fe1x and IRRI2. This differences were statistically significant by ANOVA ($F = 9.52 > 3.49$, $F = 12.73 > 3.49$ and $F = 10.74 > 3.49$, respectively for Days 14, 21 and 28). According to the Tukey test used to compare the pairs of averages (Table II), the biomass of IRRI2 was statistically different from IRRI1-Fe10x at 14 ($q = 6.803 > 4.199$), 21 ($q = 7.972 > 4.199$) and 28 days ($q = 7.455 > 4.199$). The biomass of IRRI1-Fe1x was statistically unlike that of IRRI-Fe10x at 14 ($q = 5.688 > 4.199$) and 28 days ($q = 6.111 > 4.199$). There were significant differences between IRRI2 and H-40 only on Days 14 ($q = 4.269 > 4.199$) and 21 ($q = 6.852 > 4.199$).

Discussion

The differences in type and concentration of chemical compounds of the four culture media do not seem to hinder the growth and development of *A. filiculoides*, as demonstrated by the DT and RGRs that were similar to the values obtained by Arora and Singh (2003). Although the low relative humidity (55.8%) used in the present study, differed from that of other studies (70–75%, Costa et al. 1994; 85–90%, Wagner 1997), this did not change the growth of *A. filiculoides*.

The curling of the roots has been attributed to a deficiency in phosphorus (Cohn & Renlund 1953; Lumpkin & Plucknett 1980). However, in the present investigation this seems not to be the case, because in IRRI2, with the lowest concentration of phosphorous (20 μM), the plant did not show curled roots. Hence, it is not only the deficiency of this mineral, but also other abiotic factors, as well as the proportion of the macro- and micronutrients that probably cause the root curling.

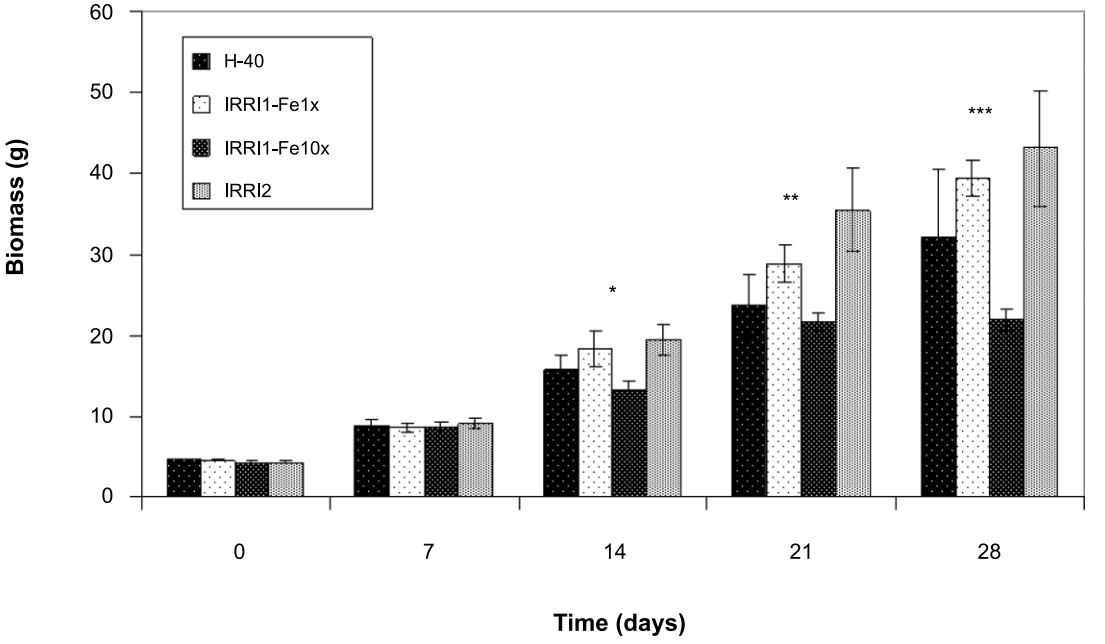

Figure 2. Biomass of *A. filiculoides* grown in H-40, IRRI1-Fe1x, IRRI1-Fe10x and IRRI2 culture media. The data show the means of four replicas; the vertical bars correspond to the standard deviation. The asterisks indicate values that are statistically significant by the ANOVA test (*p = 0.002; **p = 0.0005; ***p = 0.001).

Table II. Tukey test applied to the biomass of *A. filiculoides* growing in four artificial media under optimised conditions.

	Culture medium			
Day	H-40	IRRI1-Fe1x	IRRI1-Fe10x	IRRI2
14	15.63^{b}	18.43^{c}	13.38^{ac}	19.42^{ab}
21	23.60^{b}	28.84	21.66^{a}	35.47^{ab}
28	32.06	39.33^{b}	21.91^{ab}	43.16^{a}

Means within a line for the same day followed by the same letter are statistically significantly different.

The biomass production and RGR for IRRI1-Fe10x were the lowest, which points to a different performance of the pteridophyte in a solution with high iron concentration. A pH reduction in the medium could have made the iron unavailable for uptake by the roots (Peters et al. 1980). Maybe this effect was more severe in the medium where the iron is 10 times higher (IRRI-Fe10x). Nevertheless, the fern biomass in IRRI1-Fe10x medium was always statistically different from that of IRRI2 and IRRI1-Fe1x at Days 14 and 28. This indicates that, in the former, the macro- and micronutrient concentrations and the iron source were probably the causes for the differences; as for the last, it suggests that the higher iron concentration influenced the growth behaviour of *A. filiculoides*.

Under optimised artificial conditions (temperature, light intensity, relative humidity, pH and photoperiod), *A. filiculoides* exhibits an excellent development and growth, without necrosis. IRRI2 medium gave the lowest DT, the highest RGR and biomass, and the roots were not curled.

Acknowledgements

This work was supported by FCT-MCES under POCTI and by FSE-III QCA through the fellowship PRAXIS XXI/BD/21325/99 to A. L. Pereira.

References

Allen MB, Arnon DI. 1955. Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. Plant Physiol 30: 366–372.

Arora A, Singh PK. 2003. Comparison of biomass productivity and nitrogen fixing potential of *Azolla* SPP. Biomass Bioenerg 24: 175–178.

Carrapiço F, Teixeira G, Diniz MA. 2000. *Azolla* as a biofertiliser in Africa: A challenge for the future. Revista Ciências Agrárias 23: 120–138.

Cohn J, Renlund RN. 1953. Notes on *Azolla caroliniana*. Am Fern J 43: 7–11.

Costa ML, Carrapiço F, Santos MCR. 1994. Biomass and growth characterization of *Azolla filiculoides* in natural and artificial environments. In: Hegazi NA, Fayez M, Monib N, editors. Nitrogen fixation with non-legumes. Cairo: American University in Cairo Press. pp. 455–461.

Lumpkin TA, Plucknett DL. 1980. *Azolla*: Botany, physiology, and use as a green manure. Econ Bot 34: 111–153.

Pabby A, Dua S, Ahluwalia AS. 2001. Changes in ammonia-assimilation enzymes in response to different nitrate levels in *Azolla pinnata* and *A. microphylla*. J Plant Physiol 158: 899–903.

Peters GA, Toia RE Jr, Evans WR, Crist DK, Mayne BC, Poole RE. 1980. Characterization and comparisons of five N_2-fixing *Azolla-Anabaena* associations. I. Optimization of growth,

conditions for biomass increase and N content in a controlled environment. Plant Cell Environ 3: 261–269.

Pierik RLM. 1987. In vitro culture of higher plants. Dordrecht, The Netherlands: Martinus Nijhoff.

Wagner GM. 1997. *Azolla*: A review of its biology and utilization. Bot Rev 63: 1–26.

Watanabe I, Espinas CR, Berja NS, Alimagno BV. 1977. Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice. IRRI Research Paper Series 11: 1–15.

Zar JH. 1999. Biostatistical analysis. Upper Saddle River, NJ: Prentice Hall.