The response of *Anabaena*-free *Azolla* and the symbiotic *Azolla* to temperature

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SUMMARY

The performance of *Anabaena*-free (algae free) and symbiotic types of three species of *Azolla* (*A. filiculoides*, *A. pinnata* and *A. microphylla*) were studied in a phytotron at two average temperatures (22 and 33 °C). The growth of both the *Anabaena*-free and symbiotic types were depressed at a high temperature (33 °C) to varying degrees for all species of *Azolla* tested. There was a marked reduction of both growth and nitrogen-fixing ability (percent N) of the symbiotic *Azolla* compared to its *Anabaena*-free counterpart suggesting that the low tolerance of *A. filiculoides* No.101 to high temperature was probably dictated by the symbiont *Anabaena azollae*. *A. azollae* of both *A. microphylla* No. 418 and *A. pinnata* No. 2 appeared more tolerant to high temperature than that of *A. filiculoides* No. 101.

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RÉSUMÉ

ASUMING-BREMPONG, S. & WATANABE, I.: *La réaction de l'*Azolla *sans l'*Anabaena *et l'*Azolla *symbiotique à la température*. Les comportements de trois espèces de l'*Azolla* sans l'*Anabaena* (sans des algues) et l'*Azolla* symbiotique, à savoir, *Azolla filiculoides*, *Azolla pinnata* et *Azolla microphylla* ont été étudiés dans un phytotron à deux niveaux de la température (22 et 33 °C). Les croissances de l'*Azolla* sans l'*Anabaena* et l'*Azolla* symbiotique ont plus ou moins tombée à la temperature de 33 °C maïs pour des niveaux differents pour toutes les espèces testés. Il y a eu de la baisse plus importante du croissance et l'abilité de fixer l'azote (% N) de l'*Azolla* symbiotique que l'espèces sans l'*Anabaena*. Les résultats indiquent que l'*Azolla filiculoides* ne supporte pas bien de hauts niveaux des températures et que ce comportement est probablement déterminé par la symbiont l'*Anabaena-Azollae*. L'*Anabaena azollae* de l'*Azolla microphylla* 418 et l'*Azolla pinnata* 2 supporte bien de hauts niveaux des températures que l'*Anabaena azollae* de l'*Azolla filiculoides* 101.

Introduction

Azolla is an aquatic fern normally found floating on rivers, stagnant water ponds, etc. It habours a cyanobacteria, *Anabaena azollae* in the dorsal lobe cavity of leaves which is capable of fixing atmospheric nitrogen. *Azolla* has attracted the interest of scientists all over the world due to its fast multiplication, high nitrogen fixation rates, ability to retain nitrogenase activity in the presence of nitrogen, and easy cultivation with rice as an intercrop (Watanabe, Berja & del Rosorio, 1980; Singh & Singh, 1986). However, *Azolla* has low tolerance to high temperature and the most favourable mean air temperature for the growth of Azolla is 20 - 30 °C (Lumpkin & Plucknett, 1982). Watanabe, Espinas, Berja & Alimagno (1977) maintained an 8 °C difference between the day and night temperatures with a photoperiod of 12 h and found that the fresh weight yield and nitrogen accumulation was not significantly different among treatments at 22, 25 and 28 °C, but at 31°C, the growth of *Azolla* decreased. The heterocyst frequency also decreased in the first week at high temperature (Tung & Watanabe, 1983). *Azolla* is most susceptible to high temperature damage when the plant approaches stationary growth phase (Watanabe & Berja, 1982).

The objective of the present study was to

determine the causes for the poor performance of *Azolla* at high temperatures using both the symbiotic and *Anabaena*-free *Azolla*.

Materials and methods

Three species of *Azolla* (*A. pinnata, A. microphylla,* and *A. filiculoides*) were used. Each species consisted of both the *Anabaena*-free and the symbiotic *Azolla* strains (Table 1). The *Azolla* strains were preconditioned in the nutrient medium of Watanabe *et al.* (1977) and the *Anabaena*-free *Azolla* were grown in the same medium with 80 mg NH_4NO_3/l.

The preconditioning was carried out in a controlled cabinet (KG, Koito Kogyo Co., Japan) at the following conditions:

Relative humidity	-	75 per cent
Photoperiod	-	12 h
Light intensity	-	13 klx (at the level of grown *Azolla*)
Temperature	-	26/18 °C (average temperature 22 °C).

After the preconditioning, 0.6 g of fresh *Azolla* strains were introduced into 780 ml brown bottles (10 cm high and 10 cm diameter) containing 450 ml of the required nutrient medium. The bottles with their contents were first incubated for 4 weeks at an average temperature of 33 °C (37/29 °C, day/night temperatures). The procedure was repeated with an average temperature of 22 °C. The *Azolla* strains were thinned down to the initial inoculum rates whenever maximum biomass was attained. Samples of the *Anabaena*-free *Azolla* strains were examined before and after each experiment using the squash method (Van Hove, Diara & Godord, 1983) to ensure that they were still *Anabaena*-free.

Parameters collected included the accumulated fresh weight, dry weight, relative growth rate, percent N and total N uptake.

TABLE 1

Azolla Strains Used

Azolla *strains*	*Origin*
Anabaena-free *A. microphylla* # 427	From the germinating sporocarp of *A. microphylla* # 418 (IRRI*)
Anabaena-free *A. microphylla* # 429	From the germinating sporocarp of *A. microphylla* # 418 (IRRI)
(Symbiotic) *A. microphylla* # 418	Paraguay (IRRI collection)
Anabaena-free *A. pinnata* # 97	From the tissue culture of *A. pinnata* # 2 (IRRI)
(Symbiotic) *A. pinnata* # 2	Malaysia (IRRI collection)
Anabaena-free *A. filiculoides* # 112	From the germinating sporocarp of an unknown parent (IRRI collection)
Anabaena-free *A. filiculoides* # 131	Naturally occuring in England (IRRI collection)
Anabaena-free *A. filiculoides* # 132	Sporocarp germination of *A. filiculoides* (IRRI collection)
(Symbiotic) *A. filiculoides* # 101	German Democratic Republic (IRRI collection)

*IRRI - International Rice Research Institute, Philippines

Results

At 33 °C, *A microphylla* No. 418 produced the highest fresh weight among the symbiotic *Azolla* strains followed by *A. pinnata* No. 2 and *A. filiculoides* No. 101 (Fig. 1). In the case of *Anabaena*-free *Azolla* strains, *A. microphylla* No. 427 and 429 performed best, followed by *A. pinnata* No. 97 whilst *A. filiculoides* No. 112 and 132 performed poorest (Fig. 2). The degree of tolerance of *Anabaena*-free *Azolla* to high temperature was similar to that of the symbiotic strain of that species.

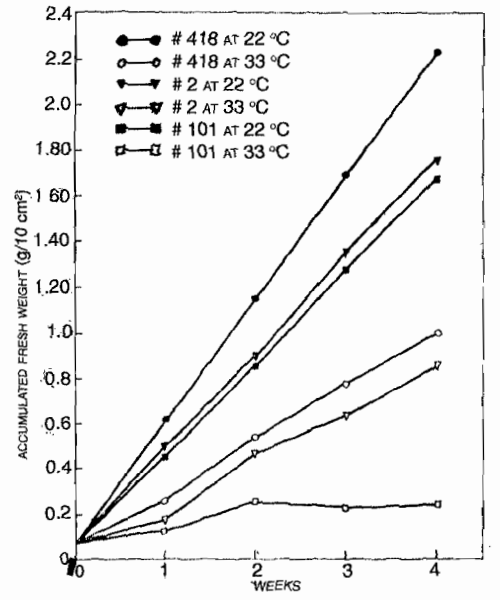

Fig. 1. Accumulated fresh weight of *A. pinnata* #2, *A. filiculoides* #101 and *A. microphylla* # 418 within 4 weeks of growth at mean temperatures of 22 and 33 °C.

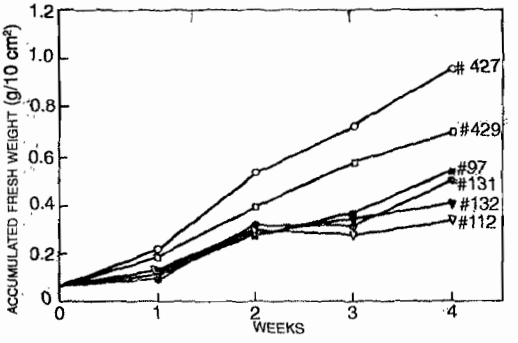

Fig. 2. Accumulated fresh weight of *Anabaena*-free ***Azolla*** strain[8] within 4 weeks of growth at mean temperature of 33 °C. # 97 - *A. pinnata*; # 112, # 131, # 132 - *A. filiculoides;* # 427 and # 429 - *A. microphylla* strains.

The difference in fresh weight at 22 and 33 °C indicates the ability of high temperature to reduce the growth of *Azolla*. The growth of *A. microphylla* No. 418 and *A. pinnata* No.2 were almost equally reduced by high temperature; however, the growth of *A. filiculoides* No. 101 was reduced by 85 per cent (Table 2). *Anabaena*-free *Azolla* of *A. pinnata* was found to be as equally tolerant to high temperature as its symbiotic strain. The algal-free fern showed a more significant tolerance to high temperature than the symbiotic ones for *A. filiculoides* and *A. microphylla* strains.

A significant correlation was obtained between the percent difference in fresh weight and percent difference in total N at 22 and 33 °C with an r^2 of 0.57 (Fig. 3). This implies that, apart from high temperature affecting the fresh weight of *Azolla* to some extent the nitrogen-fixing ability of *Azolla* is also affected. The percent N of *A. filiculoides* No. 101 dropped considerably from 5.02 per cent at 22 °C to 1.9 per cent at 33 °C (Fig. 4) whilst the drop of percent N in *A. microphylla* No. 418 and *A. pinnata* No. 2 at 33 °C was from 5.2 to 4.6, and 5 to 3.2 per cent respectively.

The drastic reduction of percent N of *A. filiculoides* No. 101 further substantiates the observation that the symbiont of *A. filiculoides* No. 101 may not be tolerant to high temperature compared to that of *A. pinnata* No. 2. Maximum relative growth rate (RGR) was attained in the 2nd week after inoculation for all the strains at 33 °C except the *A. microphylla* strains for which the maximum RGR was observed in the 1st week (Table 3). There was subsequent decrease in RGR after the maximum RGR had been attained. The attainment of maximum RGR in the 1st week for the *A. microphylla* strains suggests less adjustment to high temperature stress compared to the other species of *Azolla*.

The nitrogen fixing rate was estimated as 8.5, 3.9 and 1.5 mg N/g dry weight for *A. microphylla* No. 418, *A. pinnata* No. 2 and *A. filiculoides* No. 101 respectively at 33 °C. This estimation was based on the relative growth rate at the first week and the nitrogen content.

Discussion and conclusion

A. microphylla No. 418 was found to be tolerant to the high temperature (33 °C) whilst *A. filiculoides* No. 101 was not. The symbiont *Anabaena azollae*

TABLE 2

Mean Percent Difference in Fresh Weight of Azolla *Strains Grown at Mean Temperatures of 22 and 33 °C*

Azolla *strains*	*Mean percent difference in fresh weight at 22 and 33 °C*
Anabaena-free *A. pinnata* # 97	59.76 [bcd]
Symbiotic *A. pinnata* # 2	49.70 [def]
Anabaena-free *A. filiculoides* # 112	71.63 [a]
Anabaena-free *A. filiculoides* # 131	64.30 [abc]
Anabaena-free *A. filiculoides* # 132	67.17 [ab]
Symbiotic *A. filiculoides* # 101	84.67 [h]
Anabaena-free *A. microphylla* # 427	9.55 [g]
Anabaena-free *A. microphylla* # 429	40.07 [f]
Symbiotic *A. microphylla* # 418	55.23 [cde]

Means followed by the same letter are not significantly different from each other at 5 per cent level (DMRT).

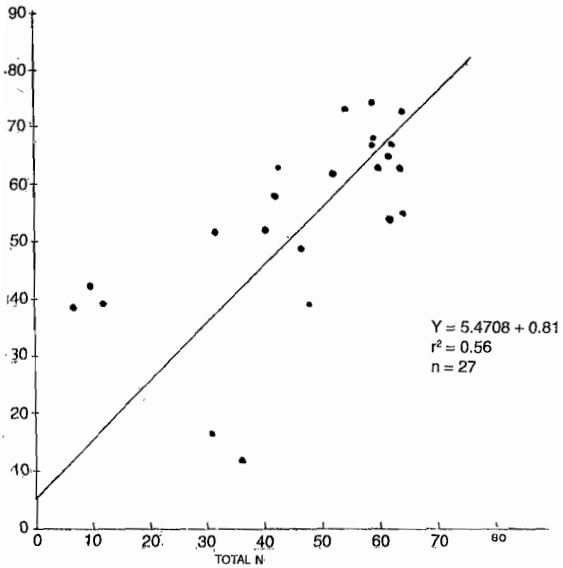

Fig. 3. Relationship between the reduction in fresh weight and total N at mean temperatures of 22 and 33 °C.

appeared to have contributed to the low tolerance of *Azolla* to high temperature based on the significant reduction in percent N of the symbiotic *Azolla* strain at 33 °C. The growth of symbiotic *Azolla* strains were also reduced as compared to the *Anabaena*-free *Azolla* strains. Ladha & Watanabe (1982) showed that *Anabaena azollae* in all species of *Azolla* shared identical and highly specific antigen and this confirmed the assumption of a single *Anabaena* species as the algal symbiont. The different ranges of heat tolerance exhibited by the various symbionts in the symbiotic *Azolla* strains used in the experiment may, therefore, be due to differences in the species of *Azolla*.

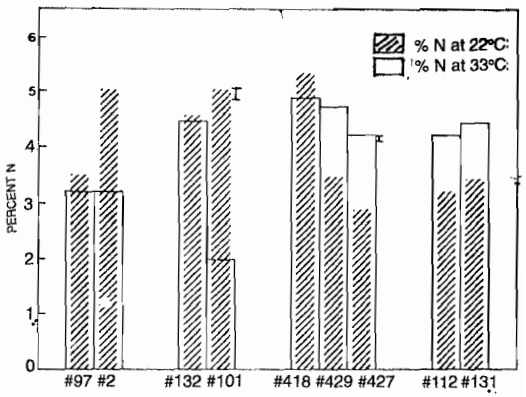

Fig. 4. Percentage nitrogen of *Azolla* strains at mean temperatures of 22 and 33 °C. # 2-*A. pinnata*; # 97 - *An*-free *A. pinnata*; # 101 - *A. filiculoides*; # 112, # 131, # 132 - *An.*- free *A. filiculoides*; # 418 - *A. microphylla*; # 427 and # 429 - *An.*-free *A. microphylla*. LSD (P=0.05) value is shown in vertical bar for 22 and 33 °C.

TABLE 3

Mean Relative Growth Rate (RGR) in g/g/d of Azolla *Strains at Mean Temperatures of 33 and 22 °C*

Strain	33 °C				22 °C			
	1st week	*2nd week*	*3rd week*	*4th week*	*1st week*	*2nd week*	*3rd week*	*4th week*
An.-free *A. pinnata* # 97	0.057 cd	0.129 ab	0.04 ab	0.54 ab	0.176 d	0.111 ab	0.56 ab	0.53 a
A. pinnata # 2	0.122 b	0.139 ab	0.044 a	0.042 a	0.269 a	0.057 b	0.57 ab	0.37 b
An.-free *A. filiculoides* # 132	0.077 c	0.106 b	0.029 a	0.025 bc	0.168 de	0.117 c	0.062 a	0.52 a
A. filiculoides # 101	0.76 c	0.094 b	0.035 c	0.0065 c	0.053 b	0.093 ab	0.056 ab	0.039 b
A. microphylla # 418	0.175 a	0.104 b	.0.053 bc	0.35 bc	0.301 a	0.087 b	0.055 ab	0.039 b
An.-free *A. microphylla* # 4 27	0.151 ab	0.129 ab	0.041 a	0.039 abc	0.148 e	0.089 b	0.036 d	0.022 c
An.-free *A. microphylla* # 4 29	0.125 b	0.109 b	0.055 a	0.027 bc	0.215 c	0.019 c	0.042 cd	0.042 b
An.-free *A. filiculoides* # 131	0.035 d	0.167 a	0.006 bc	0.069 a	0.234 bc	0.094 ab	0.047 bcd	0.042 b
An.-free *A. filiculoides* # 112	0.077 c	0.116 ab	0.008 bc	0.0825 bc	0.214 c	0.091 abc	0.052 abc	0.036 b

Means followed by the same letters are not significantly different from each other at 5 per cent level (DMRT).
An. - Anabaena

It might be that the symbiont *Anabaena azollae* of *A. filiculoides* No.101 is psychrophillic or that the nitrogen fixation mechanism is not well protected at high temperature compared to the other two symbiotic *Azolla* strains.

It could thus be concluded that the symbiont *Anabaena azollae* dictates to a large extent the degree of tolerance to high temperature in the *Azolla* species tested. For the humid tropics, *A. microphylla* No. 418 could be a promising strain.

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