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Germination of Water Fern *Azolla caroliniana* Sporocarps at Varied Light, Amino Acids, Sugars and Abscisic Acid

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The influence of light quality, amino acids, sugars and abscisic acid on the germination of *Azolla caroliniana* wild sporocarps was studied under controlled conditions in a culture room. The sporocarps incubated under blue, red, green and yellow light showed 24 to 53% higher germination than those incubated under normal light. All the amino acids tested showed a positive influence on the sporocarps germination, but glutamine and methionine were relatively more effective - giving 97 and 87% increased germination over the control, respectively. Among the sugars tested, glucose (5 to 20 mM), frutose (5 to 20 mM) and sucrose (15 and 20 mM) significantly enhanced the germination while maltose did not have any effect. At 20 mM concentration, glucose, fructose, and sucrose increased the germination by 63, 61 and 37% over the control, respectively. Abscisic acid (ABA) at 20 and 25 ppm significantly inhibited the sporocarp germination respectively by 24 and 72% over the control. The megasporocarps incubated with 30 ppm ABA did not germinate. However, at lower concentrations ABA enhanced the germination.

Key Words: **Light quality, Abscisic acid, Amino acids, *Azolla caroliniana*, sporocarp germination, Sugars**

Introduction

The water fern *Azolla* is used as a potential biofertilizer for cultivation of rice due to its ability of nitrogen fixation through the symbiotic blue-green alga, *Anabaena azollae* (Singh 1989, Lumpkin 1987, Singh & Singh 1991, Watanabe & Liu 1992). It is a heterosporous fern and forms both the micro- and mega sporocarps. The current practice of *Azolla* multiplication through vegetative propagation and its large-scale transportation is quite cumbersome. One of the options to

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overcome this problem is to develop a suitable technology for raising *Azolla* plants from the sporocarps which would also be helpful in the maintenance of *Azolla* germplasm. Further, storage, transportation and distribution of the sporocarps are easier and more economical. The sporocarps of *A. filiculoides* are reported to have been used successfully for application in the rice fields in China (Shuying 1987). However, information on the germination of *Azolla* sporocarps is rather limited. Studies on the factors influencing sporocarp germination are desired for developing suitable methods for ensuring maximum germination. Singh et al. (1990) have reported the effects of different nutrient media, *p*H, phosphorus, nitrogen and growth hormones on the germination of *A. caroliniana* sporocarps. The present study describes the influence of light quality, amino acids, sugars and abscisic acid on the germination of sporocarps in this species.

Materials and Methods

Collection of Sporocarps

Azolla caroliniana wild was selected for this study due to its better growth, nitrogen fixation and tolerance to insect pests and diseases, as compared to the native *A. pinnata* strains (Singh et al. 1991). The sporulation in this species occurs at Cuttack during December to February. The plants bearing sporocarps were sun-dried and powdered. The dried *Azolla* powder was vigrously shaken in a tray and balls of megasporocarps with attached masullae were collected through a series of sieves as described by Singh et al. (1990).

Germination of Sporocarps

The sporocarps were first soaked in sterile water for 24 hr, surface sterilized with 0.01% mercuric chloride and then washed repeatedly in sterile water. Subsequently, these were spread as monolayer in Petri dishes containing sterilized N-free IRRI medium composed of P (20 ppm), K^+ (40 ppm), Ca^{++} (40 ppm) Mg^{++} (40 ppm), Mn^{++} (0.5 ppm), B^+ (0.1) ppm, Mo (0.1 ppm), Zn 0.01 ppm, Cu^{++} (0.01 ppm) and Fe^{+++} (2 ppm). Plates were then sealed with cellophane tape and incubated in a culture room at 24 ± 2°C under 2.5 K lux whilte fluorescent light (16 hr light/8 hr dark). The effect of different light quality on the germnation of sporocarps was studied by covering the petridishes with yellow, red, green, blue and black transparent plastic films (table 1). The uncovered petridishes served as the control. To study the effect of various chemicals on

Table 1 *Effect of light quality on germination of Azolla caroliniana sporocarps*

Treatments	No. of sporo-carps incubated	No. of sporo-carps germinated	Germi-nation in angular value
No light (dark)	106	0	0.0
Normal light (control)	119	57	43.8 (47.9)
Yellow light	91	54	50.4 (59.3)
Red light	120	79	54.2 (65.8)
Green light	113	72	53.0 (63.7)
Blue light	112	82	58.8 (73.3)
CD 5%			5.0
1%			7.1

Figures in parentheses indicate the % germination the germination, nutrient medium was supplemented with different amino acids (CDH, India) at 100 ppm each (table 2), glucose, fructose, sucrose and maltose (Himedia, India) at 2.5 to 20 mM each (table 3) and abscisic acid (Glaxo lab, India) at 5 to 30 ppm (table 4). The experiments were laid out in a complete randomized design with three replications and each experiment was repeated twice. The number of germinated sporocarps was counted 20 days after incubation and germination per cent was calculated. The data were transformed to angular for the purpose of statistical analysis and critical difference (CD) at 5% and 1% probability levels were calculated. The results are discussed on the basis of CD at 5%.

Table 2 *Effect of different amino acids on germination of Azolla carolinian sporocarps*

Amino acids	No. of sporocarps incubated	No. of sporocarps germinated	Germination in angular value
Control	83	33	39.1 (39.7)
Methionine	78	58	59.5 (74.3)
Cystine	90	61	55.4 (57.8)
Glutamine	74	58	62.3 (78.4)
Adenine	85	56	54.3 (65.9)
Alanine	86	56	53.8 (65.1)
Proline	80	53	54.5 (66.2)
Valine	96	63	54.1 (65.6)
Histidine	75	48	53.1 (64.0)
Trytophane	86	60	56.7 (69.8)
CD 5%			3.4
1%			4.7

Figures in perenthesis indicate the % germination

Results and Discussion

Effect of Light

Light is known to promote the germination of seeds in many plant species through termination of dormancy. However, different species require distinctly different photoequilibrium values, which are satisfied at different spectral compositions (Bewley & Black 1982). The present study showed that the sporocarps incubated in dark did not germinate, confirming the reports of Qing-yuan et al. (1987) and Singh et al. (1990) that light was a prerequisite for their germination. The megasporocarps incubated under blue, green, red and yellow light showed 24 to 53% higher germination than those incubated under normal light (table 1). The percentage of germination was the highest under blue light, which was closely followed by red light. The germination under yellow and green light was significantly lower than under blue light. Koller et al. (1964) reported that germination of *Artimisia monosperma* achenes was stimulated by light in all regions of the visible spectrum and suggested that in this species phytochrome was either absent or masked by a different pigment which absorbed light in the entire visible spectrum. A similar mechanism might be operative in *Azolla* also, but this needs confirmation.

Table 3 *Effect of different sugars on germination of Azolla caroliniana sporocarps*

Concentration (mM)	No. of sporocarps incubated				No. of sporocarps germinated				Percentage germination in			
	Glucose	Fructose	Sucrose	Maltose	Glucose	Fructose	Sucrose	Maltose	Glucose	Fructose	Sucrose	Maltose
0.0	112	112	112	112	54	54	54	54	44.0 (48.2)	44.0 (48.2)	44.0 (48.2)	44.0 (48.2)
2.5	116	117	106	112	58	60	56	54	45.0 (50.0)	45.8 (51.3)	46.6 (52.8)	44.0 (48.2)
5.0	110	107	119	110	61	63	62	55	48.1 (55.4)	50.1 (58.9)	46.2 (52.1)	45.0 (50.0)
7.5	107	114	112	109	61	77	61	55	49.0 (57.0)	55.2 (67.5)	47.6 (54.5)	45.2 (50.4)
10.0	123	104	113	107	75	75	59	55	51.4 (61.0)	58.1 (72.1)	46.3 (52.2)	45.8 (51.4)
15.0	110	108	110	105	85	70	61	53	61.6 (77.3)	59.4 (74.1)	48.1 (55.4)	45.3 (50.5)
20.0	116	115	115	109	91	89	76	59	62.3 (78.4)	61.6 (77.4)	54.5 (66.1)	47.4 (54.1)
CD											5%	1%
Sugars											1.5	2.0
Concentrations											1.9	2.6
Sugars x Concentrations											3.9	5.2

Figures in parentheses indicate the % germination

Effect of Amino Acids

All the amino acids tested showed a significant positive influence on the germination of the sporocarps (table 2), possibly due to their direct involvement in protein synthesis. Further, amino acids are also used in respiratory metabolism via krebs cycle after being converted to keto acids through deamination and transamination reactions (Collins & Wilson 1975). Glutamine recorded about two-fold germination per cent as compared to the control. Methionine recorded slightly lower percentage of germination than glutamine but the difference was not significant. The percentage of germination was the lowest with histidine, which was at par with alanine, valine, adinine, proline and cystine. Glutamine and glutamate are reported to be the major contributors of nitrogen for synthesis of nitrogenous compounds in the developing seedlings (Bewley & Black 1982) The beneficial effects of certain amino acids on the germination of *A. mexicana* sporocarps was reported earlier (Kannaiyan 1988).

Effect of Sugars

Of different sugars tested, glucose, fructose and sucrose significantly increased the germination over the control (table 3) possibly due to their utilisation as respiratory substrates by the germinating sporocarps. However, maltose had no effect on the sporocarp germination. This suggests that *Azolla* sporocarps might be lacking in maltose-hydrolyzing enzymes. Glucose and fructose were more effective than sucrose, which has to be hydrolyzed to monosaccharides before being used in the respiratory metabolism. Increasing the concentration of glucose and fructose from 5 to 20 mM gradually enhanced the sporocarp germination by 15 to 63% and 22 to 61% over the control, respectively. At 7.5 and 15 mM concentrations, fructose showed significantly higher percentage of germination by 15 and 37% over the control, respectively. However, at lower concentrations it did not affect the germination significantly.

Effect of Abscisic Acid

The data showed that abscisic acid (ABA) at 20 and 25 ppm reduced the germination significantly by 24 and 72% over the control, respectively (table 4). The sporocarps incubated with 30 ppm ABA did not germinate. The inhibition of seed germination due to ABA is reported in many plant species (Bewley & Black 1982). Karssen (1976) reported that exogenous application of ABA reduced the germination of light-requiring *Chenopodium album* seeds through inhibition of radicle growth, but it did not affect the light-dependent termination of seed dormancy and induction of germination. However, ABA at 5 to 15 ppm enhanced the sporocarp germination, — percentage of germination being the highest at 10 ppm. The reasons for the increased sporocarp germination at lower concentrations of ABA are not clearly understood. However, small amount of ABA is reported to enhance the growth and nitrogen fixation of developed *Azolla* sporophytes (Cadiz & Alegar 1986).

Table 4 *Effect of abscisic acid on germination of Azolla caroliniana sporocars*

Abscisic acid (ppm)	No. of sporocarps incubated	No. of sporocarps germinated	Percentage germination in angular value
0	122	52	40.7 (42.6)
5	124	91	59.0 (73.4)
10	120	106	70.0 (88.3)
15	124	80	53.4 (64.5)
20	142	46	34.7 (32.4)
25	119	14	20.1 (11.8)
30	108	0	0.0
CD 5%			5.1
1%			7.2

Figures in parentheses indicate the % germination

The studies indicated that light was absolutely necessary for the germination of *Azolla coroliniana* sporocarps and quality of light had a significant effect on the rate of their germination. Amino acids and certain sugars enhanced the sporocarp germination, while abscisic acid at high concentrations (20 ppm or more) inhibited the germination.

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