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Regeneration of *Azolla pinnata*

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The size of frond tissue of *Azolla pinnata* had a differential effect on the regeneration capacity. The branches that consisted of large number of fronds had a greater influence than branches having fewer fronds. Two fronds in a cutting were essential for regeneration into an individual. The conspicuous presence of a root in large branches with higher regeneration might have a role to some extent. Green fronds had more influence than pinkish fronds. Application of phosphorus augmented the regeneration capacity, particularly in pinkish fronds. The overall regeneration capacity of a plant was more after segmentation into pieces compared with intact individual and this was stimulated by phosphorus.

Azolla pinnata has been recognised as biofertilizer throughout the world (Moore, 1969, Watanabe, 1982). The alga, *Anabaena azollae* that harbours in each frond of *Azolla* fixes atmospheric nitrogen symbiotically and remains present during all the stages of frond development. The plant propagates vegetatively as well as sexually but vegetative propagation is more prolific. Light plays a major role in governing the plant growth and its regeneration. Strong sunlight displays red colouration whereas in the shade, they remain green (Lumpkin & Plucknett 1980). The optimum pH for growth is neutral (Levan & Sobhachkin, 1963). The deficiency of some nutrients, particularly P is more marked on growth and nitrogen fixation (Subodhi and Singh, 1978; Watanabe, 1982). But there is no report as to the size of the vegetative tissue responsible for its regeneration into an individual. The objective of this study was to analyse the effect of size of frond tissue on the regeneration of plants having diverse environmental stress.

MATERIALS & METHODS - A full size mature plant has 7 branches and is capable of separation into an individual plant from the mother plant due to pressure of wind or current at the abscission layer. The full size mature plant of Bangkok isolate *A. pinnata* was used. The branches were carefully segmented into seven pieces according to the serial number of branches from apex to base. The pieces were collected from the green stock as well as from pinkish stock. The size (sq. mm) of the mature plant and the branches from apex were the following:- mature plant - 27*,

1.1 2.2 3.25 4.3 5.5 6.7 7.8

One ml of 1% triple superphosphate was carefully applied with brush on the leaves of ten mature plants 24 h before excision of the branches. Branches in order of maturity from apex to base include 1, 2, 3, 4, 5, 6, 7 fronds; each frond having the dorsal and ventral lobes so that the largest branch contains 7 fronds and the smallest apical branch only one frond having one hyaline ventral lobe and one green dorsal lobe. The branches are without roots excepting the largest one having solitary root. The mature plant of each of the green and pinkish stocks includes single root, 31 fronds and 7 branches. The experiments were conducted in March-April in small earthern pots containing 250 ml tap water. The level of water was regularly maintained. All sets were kept in open sunlight in net house of the department. The data were recorded daily to observe the initiation of roots and to measure the number of roots, length of roots, number of branches, number of fronds, and the successful branches that survived. The final data were taken

after 5 days, taking average of 20 cuttings. The time to attain the mature stage of each of the fragmented cuttings was noted carefully after attainment of 7-branch mature state upto 25th day from the date of setting the experiment. Control sets from both green and pinkish stock were maintained side by side.

RESULTS The individual branches of the mature plant showed differential growth and potentiality of regeneration. The 7th branch of green plants showed more vigour and vitality and it quickly regenerated into its original parent form. The growth rate of each branch increased according to the number of fronds present in the branches. The survival capacity was 100 percent in all branches excepting the first one where it was only 20 percent and most of the branches were degenerated within 3 days. It was as late as 10 days in 2nd branch. The 7th branch took only 8 days to have mature state whereas the 6th branch took as many as 16 days to attain full size of the plant. The growth of pinkish plant was, in contrast, retarded in all respects. The survival capacity was less in smaller branches. The 7th branch required 12 days to attain maturity whereas the 6th branch needed 22 days (Table 1).

The application of phosphorus in the form of triple superphosphate showed stimulating effect, particularly in pinkish plants. The number of fronds in green cuttings increased from 48 to 59 and the branches increased from 12 to 17 only. The survival capacity increased from 20 to 60 in the first branch. Due to enhanced growth rate, the green cuttings of 7th branch, relatively quickly, attained the full size, 2 days earlier due to the application of phosphorus. This stimulating effect of phosphorus was pronounced in pinkish plants. Phosphorus increased in number of fronds, branches and survival capacity was higher. The time required to attain the full size of 7th branch after treatment was only 9 days in contrast to 12 days from untreated ones (Table 2). When the total growth of all plants in treated and non-treated conditions was considered, the results were more interesting. Segmentation of branches showed the enhanced photosynthetic activity and the number of fronds increased by about 15-25 percent, so that the total number of fronds in segmented branches of a green plant was 48 as compared to 42 in intact plant; the same trend was observed in pinkish type also where the number of fronds in segmented branches was 39 against only 32 in intact plant. Phosphorus treatment showed ìless effect on intact green plant as indicated by 46 and 42 fronds in the treated and non-treated plants respectively; however stimulating effect occurred on treatments, especially on cuttings.

DISCUSSION - The potential regenerating capacity of cuttings of a plant that is usually vegetatively propagated, is conditioned by the internal and external factors that may not be conducive to the expression so that tissue may or may not be grown into an individual. *A. pinnata* is usually a vegetatively propagated plant. The vegatative propagation can be influenced by the size of frond tissue, represented by their number. The individual branches of the plant having varying size of frond tissue had a differential effect on the regeneration capacity. The branches that consisted of large number of fronds had a greater influence. Hence the basal branches consisting of more fronds were quickly and more frequently regenerated into an individual. Regeneration of root might have a direct influence on the regeneration of the individuals as the larger branches had a conspicuous presence of roots. The same trend was observed in cuttings from pinking stocks though the cuttings from green stock had a determining influence. At least 2 fronds in a cutting having two dorsal lobes and two ventral lobes were essential for the cutting to regenerate, of course, with difficulty. Application of phosphorus in the form of superphosphate augmented this regenerating capacity, particularly in cuttings from pinkish

Table 1 Effect of size of green (G) and pinkish (P) frond tissue on the regeneration of plants after 5 days of segmentation .

Parameter		Branches from apex to base of the plant						
		1	2	3	4	5	6	7
No. of roots	G	-	-	-	1	2	2	2
	P	-	-	-	1	1	1	2
Length of roots (mm)	G	-	-	1	12	14	14	15
	P	-	-	-	6	6	10	14
No. of fronds	G	1	4	5	6	8	9	15
	P	-	2	4	6	7	8	12
No. of branches	G	1	1	1	1	1	2	5
	P	-	1	1	1	1	2	3
Percent success	G	20	100	100	100	100	100	100
	P	-	60	60	100	100	100	100
Days to attain maturity	G	-	16	16	13	11	10	8
	P	-	22	20	16	14	14	12

Table 2 Effect of size of green (G) and pinkish (P) frond tissue on the regenertion of plant after 5 days of segmentation in phosphate treated plant.

Parameters		Branches from apex to base of the plant						
		1	2	3	4	5	6	7
No. of roots	G	-	-	1	1	2	2	2
	P	-	-	1	1	1	1	2
Length of roots (mm)	G	-	-	15	15	18	18	20
	P	-	-	10	10	20	20	20
No. of fronds	G	3	5	6	7	9	12	17
	P	3	3	5	7	9	11	16
No. of branches	G	1	1	2	2	2	3	6
	P	1	1	2	2	2	3	5
Percent success	G	60	100	100	100	100	100	100
	P	60	60	80	100	100	100	100
Days to attain maturity	G	15	15	13	11	8	8	6
	P	19	17	17	14	13	11	9

stock. This might be due to preferential role of phosphorus in growth and nitrogen fixation (Moore, 1969; Watanabe, 1982) and the presence of *A. azollae* in each dorsal lobe of a frond during all stages of frond development (Ashton & Walmsley, 1976). The overall regeneration capacity with respect to a number of plants was more in segmented cuttings than in intact individuals. In the cutting due to higher regenerating capacity the young leaves were again stimulated by phosphorus. The high metabolic rate and the symbiotic nitrogen fixation necessarily fulfill the high phosphate requirement. Morover, the young leaves contained health filaments compared with the old leaves and green fern always contain more phycocyanin pigment in the algal cells whereas algal cells of brownish plants appeared to contain more phycoerythrin (Hill, 1975). The decline in nitrogen fixation and growth of Azolla due to fragmentation of fronds by wind has been observed by Ashton & Walmsley (1976), Keller & Goldman (1979). According to Ashton (1974) plants entering the Hendrick Verwoerd Dam in South Africa through tributary streams become fragmented by wind and wave action and usually perished. In contrast the fragmented propagules were cultured in calm and still water.

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