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STUDIES ON THE EFFECTS OF EXTRACT OF *HYPNEA MUCIFORMIS* LAMOUR ON GERMINATION AND SEEDLING MORPHOLOGY IN *ARACHIS HYPOGAEA* VAR. *VRI 2*

V. JAYACHANDRAN* and V. RAMASSAMY

Department of Botany, Bharathidasan Govt. College for Women, Pondicherry-605 003, India.

* Department of Botany, Tagore Arts College, Pondicherry-605 008, India.

The present study brings out the effects of seven different (1, 2.5, 5, 7.5, 10, 15 and 20%) concentrations of extract of *Hypnea muciformis* Lamour (red alga) on *Arachis hypogaea* Var. *VRI 2*. Experiments were conducted in both petridishs and earthern pots. The effects of extract on germination percentage, shoot and root length, number of leaves and lateral roots have been brought out. In addition the effects of extract on the frequency of root nodules and epidermal morphology have also been discussed with reference to pot experiments.

Keywords : *Arachis hypogaea*; Extract; *Hypnea muciformis;* Seedling morphology.

Introduction

There are a few reports on the effects of seaweed extracts[1-5] on morphology of plants such as germination of seeds, root and shoot lengths, number of lateral roots and leaves of seedlings. However, their effects on epidermal morphoogy is altogether lacking. Similarly, their effects on the root nodules is not known so far. Therefore, an attempt has been made to study the effects of extract of *Hypnea muciformis* on the epidermal morphology and frequency of root nodules in addition to its effects on other morphological characters of seedlings.

Materials and Methods

The alga *Hypnea muciformis* Lamour was collected during the month of January 1998 from the estuary of Pannithittu, Pondicherry[6]. They were washed thoroughly in sea water and followed by tap water. They were shade dried for five days and followed by oven drying at 65°C for 12 hours. The dried algal materials were crushed to powder in a mortar and was stored in polythene bags. One litre of distilled water was added to 100 gm of algal powder and the preparation was autoclaved under 15 lbs pressure for 15 minutes. The solution was filtered while it was hot with a cheese cloth and the filtrate was treated as stock solution (100%). Different concentrations of extracts (1, 2.5, 5, 7.5, 10, 15 and 20%) were prepared immediately.

The seeds of *Arachis hypogaea* var *VRI 2* was procured from Oil Seed Research Station, Tindivanam, Tamilnadu. Twenty five seeds were soaked in each concentration (equal volume) for 12 hours. A control was maintained by soaking one set of 25 seeds in breaker containing equal volume of distilled water. Both control and treated seeds were sown in uniform earthern pots filled with garden soil. Similarly another set was maintained after soaking the seeds in extract in petridishes with moist filter papers. Triplicates of the experiments were maintained. The earthern pots were given 100 ml of repective concentration of extracts every day and in the case of petridishes 10 ml every day. Controls were given only distilled water. Readings were taken on fifth day in petridish experiments and 15th and 30th day in earthern pots. Epidermal peels were obtained uniformly from the fourth leaf of the treated and control of pot experiment seedlings by mechanical means and were stained with aqueous safranin (1%). They were mounted in 50% glycerine and sealed with DPX.

Observations

Tables 1 and 2 show the results obtained in both petridish and pot experiments. *Petridish experiments* : Cent percent germination was recorded in 7.5 and 10% concentration after 24 hours. However, cent percent germination was noticed in other concentration after 48 hours (3rd day), length of roots and shoots have become progressively decreased with increase in concentration of extract. However, more number of lateral roots were noticed in 10% concentration over control and other concentrations. As many as seven leaves were seen in plants growing in 2.5% concentration as against four leaves in control. The seedling growth appears to be poor in the higher concentration (15 and 20%) and showed symptoms of gradual decay.

Pot experiments : Seeds started germinating on 4th day. A maximum germination (67%) of seeds was observed in 10% concentration among the treated seeds and the control on 5th day. Very low concentrations (1 and 2.5%) and very high concentrations (15 and 20%) inhibited germination. Highest number (seven leaves) was found in 7.5% concentration as against six in control (Table 1). Though the control seedlings showed more number of lateral roots, the treated seedlings showed more number of root nodules.

Epidermal cells (Non-costal) of both surfaces are large, sometimes small, irregularly distributed, anisodiametric, sometime tending to become isodiametric; walls thin and arched or slightly sinuous. Stomata are sub-circular to oval, irregularly distributed, found on both surface (epiamphistomatic) (Table 2). The adaxial leaf epidermis showed a marked decrease in epidermal cell size but an increase in stomatal size and freqency of stomata, epidermal cells and oil globules. The abaxial surface showed an increase in the size and frequency of epidermal cells and frequency of stomata and a decrease in size of stomata and frequency of oil globules. Of the three stomatal types viz. para-, aniso- and tetracytic encountered in the control, paracytic ones were predominant (Table 1). In the leaves of treated seedlings tetracytic ones were altogether absent in both the surfaces except 1% and 2.5%. An increase in the frequency of paracytic and a decrease in anisocytic stomata were observed in the adaxial surface of the leaves of treated seedlings with increase in concentration of extract whereas a reverse condition is seen in the abaxial surface (Table 2). Stomatal abnormalities such as persistent stomatal initials and stomata with single guard cell were infrequently seen in all control and the leaves of treated seedlings. The former was frequent than the latter (Fig. 1).

Discussion

Several studies have been made by various workers on the effects of both fresh water[7-11] and marine[2-4] algal extract. In general the reported beneficial effects

Table 1.

SL No	CONCENTRATION %	PETRIDISHES						POTS							
		GERMINATION % DAYS AFTER		ROOT LENGTH	SHOOT LENGTH	NO. OF LEATERAL ROOTS	NO. OF LEAVES	GERMINATION % DAYS		NO. OF LEAVES DAYS		NO. OF LAT. ROOTS DAYS		NO. OF ROOT NODULES DAYS	
		1ST	2ND					5TH	10TH	15TH	30TH	1 5TH	30TH	15TH	30TH
1	CONTROL	90	100	5.4	2.5	20	4	66	66	5	6	43	39	13	20
2	1%	90	90	4.3	2.1	16	4	0	11	3	5	12	36	12	14
3	2.50%	80	100	3.1	1.7	16	7	0	11	4	5	0	28	0	10
4	5%	80	100	2.1	1.1	9	2	0	45	5	7	24	34	9	16
5	7.50%	100	100	2.4	1.8	16	0	22	56	5	7	34	38	19	26
6	10%	100	100	2	1.2	25	0	67	78	5	7	38	46	16	26
7	15%	30	100	2	1.1	15	0	56	56	3	6	34	47	13	21
8	20%	0	100	0.7	0.6	0	0	33	44	3	5	15	29	9	9

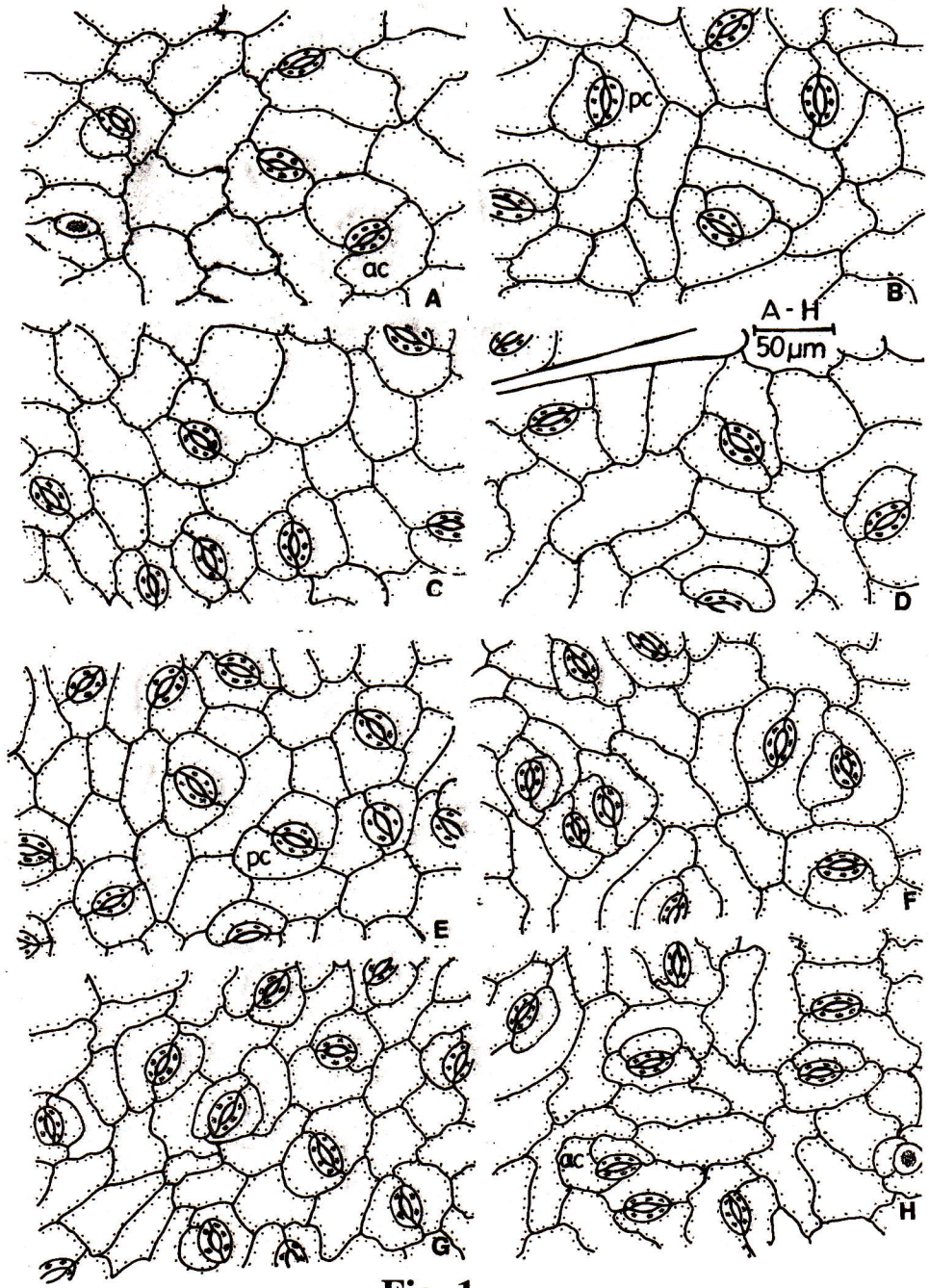

Fig. 1

Fig. 1 A-H. Leaf epidermis. A, C, E, G, Adaxial side.
B, D, F, H, Abaxial side. A, B. Control.
C. D. 7.5%. E. F. 10%. G. H. 1.5%.
(ac, anisocytic stoma; pc, paracytic stoma; tc, tetracytic stoma)

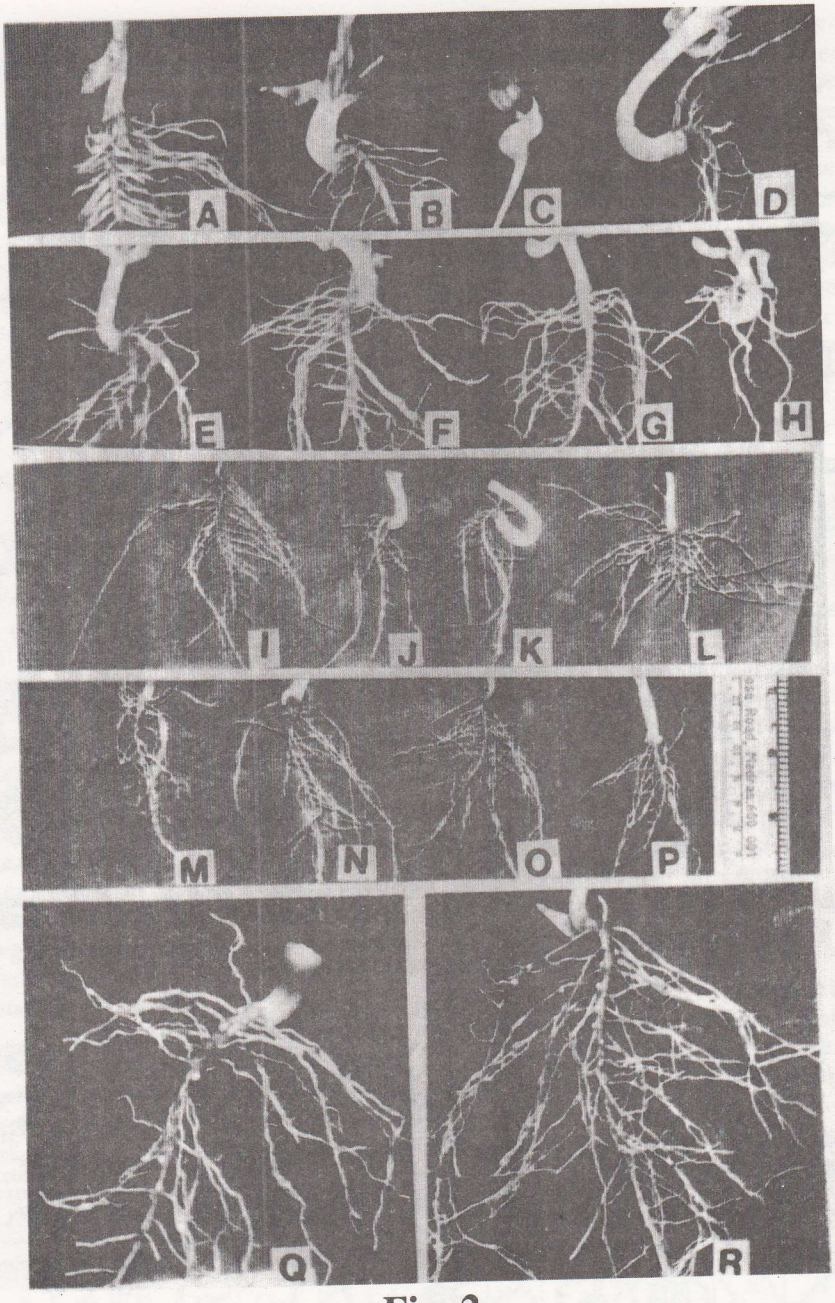

Fig. 2

Fig. 2 A - R. Root morphology and frequency of root nodules.
A - H. 15th day. I - R. 30th day. A, I. Control.
B. J. 1%. C. K. 2.5%. D. L. 5%. E. M. 7.5%.
F. N. 10%. G. O. 15% H. P. 20%. Q. 7.5% enlarged.
R. 10% enlarged (Note the prominant root nodules)

Table 2.

Concentration of Exract	Epidermal cells		STOMATA						TYPES IN %						FREQUNCY/mm² OF OIL GLOBULES	
	Size		Frequency		Size		Frequency		PARA		ANISO		TETRA			
	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB
CONTROL	45 x 22 ± 1.1	40 x 16 ± 2.2	943 ± 2	823 ± 3.6	21 x 14 ± .3	29 x 18 ± .4	122 ± .5	102 ± .9	93 ±	95	6	3	1	2	129 ± 1	189 ± 1.2
	± .7	± 1.1			± .4	± .4	±									
1%	47 x 18 ± 1.2	38 x 18 ± 1.3	961 ± 3	826 ± 2.6	23 x 15 ± .3	26 x 17 ± .2	129 ± .5	92 ± .3	92 ±	85	8	3	-	2	212 ± 1.7	181 ± .5
	± .9	± .8			± .3	± .2	±									
2.5%	26 x 18 ± 1.1	46 x 17 ± 1.3	1232 ± .8	1052 ± .9	23 x 16 ± .4	26 x 16 ± .2	162 ± .8	135 ± .9	94	90	4	10	2	-	262 ± .9	181 ± 2.0
	± .6	± .9			± .3	± .4	±									
5%	51 x 16 ± 1.4	47 x 16 ± 1	1307 ± .7	1060 ± .4	22 x 14 ± .3	25 x 14 ± .3	171 ± .7	124 ± .4	95	96	5	4	-	-	187 ± 1.3	160 ± 1.4
	± .4	± .3			± .3	± .4	±									
7.5%	40 x 19 ± 1.3	45 x 16 ± 1.3	1281 ± .9	1009 ± .6	21 x 16 ± .3	25 x 17 ± .3	180 ± .9	142 ± .6	93	92	7	8	-	-	233 ± 1.9	133 ± 1.6
	± .6	± .3			± .3	± .1	±									
10%	39 x 21 ± .9	46 x 16 ± 1.8	1329 ± .4	1011 ± .4	21 x 14 ± .3	24 x 15 ± .3	158 ± .4	136 ± .4	97	95	3	5	-	-	242 ± 1.5	180 ± 1.8
	± .6	± 1			± .3	± .3	±									
15%	46 x 18 ± 1.1	46 x 14 ± 1.2	1419 ± 1.7	1106 ± 1.3	19 x 15 ± .3	23 x 16 ± .3	181 ± 1.7	145 ± 1.3	98	91	2	9	-	-	212 ± .6	144 ± .5
	± .4	± .9			± .2	± .4	±									
20%	39 x 18 ± 1.4	39 x 16 ± 1	1196 ± .5	993 ± .9	19 x 15 ± .2	24 x 17 ± .3	154 ± .5	122 ± .9	94	84	6	16	-	-	201 ± 1	169 ± 1.3
	± .2	± .6			± .3	± .4	±									

of sea weeds are 1. Overall plant vigour[12] 2. Yield quality[13]. The present study also account for the better vegetative growth at the level of 2.5 concentration and 7.5 to 10% concentration of extract in petridish and pot experiments respectively (Tables 1 and 2).

Though there are a few works available on the effects of different seaweed extracts on pulses such as green gram and black gram[1,4,5], winged beans[3], the effect of extracts on the root nodules have not been reported. The present study reveals (Table 1) the effects of extract on its frequency. It is clear that the root nodules were found to be higher in 7.5 and 10% concentration when compared to the control. Though the frequency appears to be slightly higher, the size of the nodules are considerably larger and pinkish in colour (Fig. 2) than that of control.

The present study for the first time brings out some interesting observations on the effect of seaweed extract on foliar epidermis. Differential effect of seaweed extract was observed on different leaf surfaces. The adaxial leaf surface showed a sharp decrease in epidermal cell size but an increase was noticed in stomatal size and frequency and frequency of epidermal cells and oil globules. The abaxial surface showed an increase in the size and frequency of epidermal cells and frequency of stomata. However, the size of the stomata and frequency of oil globules have been reduced. Of the three stomatal types, para-, aniso-, and tetracytic, paracytic ones were dominant in the conrol and leaves of treated seedlings. In the leaves of treated seedlings tetracytic ones are altogether absent in both surfaces except 1 and 2.5% (Table 2). Interestingly, the other stomatal types viz. para- and anisocytic show difference in their frequency on different surfaces with increase of concentration (Table 2). It is evident that higher concentration of extract had its effect even at the level of ontogeny of stomata. However, the frequency and types of stomatal abnormalities have not been affected by the treatment.

Many workers have reported the occurrence of hormone like substances such as IAA from *Laminaria agardhii*[14], gibberellins from *Fucus vesiculosus*[15] and cytokinin from seaweed extracts[16]. The present work also suggest that the presence of certain growth hormones, extracellular products and vitamins in the extract might be reponsible for these effects.

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