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Original Research Paper

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MICROMORPHOLOGY, ULTRASTRUCTURE AND MOLECULAR STUDIES OF SEAWEED LIQUID FERTILIZER TREATED *ARACHIS HYPOGAEA* (L.)

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ABSTRACT

Study was made on the effect of seaweed liquid fertilizer (SLF) from the brown alga *Sargassum polycystum* on the germination, growth, yield and biochemical constituents of *Arachis hypogaea*. The maximum germination percentage, growth, pigments and biochemical constituents were observed in 25% concentration of SLF. The low values for seed germination, growth and yield parameters, pigments and biochemical constituents were recorded at 100% concentration of seaweed liquid fertilizer. The present study revealed that SLF from *S. polycystum* can be used for the seed treatment to increase the growth and yield of *Arachis hypogaea*. Transmission electron microscopic studies were made on the leaf cells in *Arachis hypogaea* showed that the cells were formed from the inwardly from the central cell a large inclusion was anchored to the cell wall. This inclusion was highly vacuolated containing many electron opaque matrix, some of the vacuoles appeared to be partially osmiophilic, although these more likely represent artifacts due to glancing section of the vacuole membrane. The initial stages of vacuole formation are characterized by the presence of smooth surfaced electron dense vesicles 0.1-1.0 mm, which are disposed throughout the cytoplasm continuities between smooth ER like cisterna and the enlarging vesicles suggest that the electron dense material is reduced in the abundant PER and sequestered in the smooth ER (SER) continued deposition of the cisternal material and enlargement of inter connected cisternal results in vescile coalescence to form the developing vacuole. Ultrastructural studies revealed that, originating from gland cells in comprised of numerous electron translucent vacuoles enclosed by an electron-opaque matrix. Gland cell walls are relatively thin, which in turn would aid the transfer of metabolites to the stalk-like structure. These features of the gland cells provide essential clues to the production and storage of the halogenated metabolites in *Arachis hypogaea*. High quality DNA extractions are a prerequisite for genetic studies for a variety of plants including *Arachis hypogaea*. Nowadays, there are great number of plant DNA extraction method and commercially available extraction kit are also becoming more and more popular. It appears that different procedures work best for different plant groups. Thus in the genetic studies of *A. hypogaea*, choosing CTAB method to choose becomes a concern. The DNA extracted by this method from (Control and Seaweed Liquid Fertilizer (SLF) treated fresh young leaf tissue of *A. hypogaea* was analysed according to their cost and time, yield, purity, integrity and PCR (Polymerase Chain Reaction) based downstream analysis. The quality and quantity of isolated DNA was measured by Nano photometer. The absorption value A260/280 was calculated. Based on OD values the 10% and 25% of SLF treated plants showed pure and contaminant free DNAs when compared to control plants. Further the isolated genomic DNA was checked with 0.8% agarose gel electrophoresis stained with ethidium bromide to check the DNA quality. The gel was photographed under gel documentation system. In addition, the quantity and quality of the DNA extracted by this method were high enough to perform hundreds of PCR based reactions.

KEYWORDS : Micromorphology, Ultrastructure, DNA purification, Nano photometer, PCR, extractions.

INTRODUCTION

The chemical fertilizers deteriorate soil quality and the homeostasis of ecosystem. The toxic chemicals (arsenic and cadmium), from the chemical fertilizer accumulate in plants and plant products causing health problems to humans by biomagnifications (Hansra, 1993). Therefore, the current trend is to explore the possibility of supplementing chemical fertilizer with organic manures particularly the seaweed. In India, the farmers are now advised to use the seaweed fertilizer as an alternate source to the synthetic fertilizer (Hong *et al.,* 2007). Because of auxin, gibberellin, cytokinins, betanins, macro and micronutrient contents of seaweed, the liquid extract of seaweeds are traded commercially as liquid fertilizers and biostimulants (Wu *et al.,* 1997; Durand *et al.,* 2003; Strik *et al.,* 2004; Khan *et al.,* 2009). Seaweed extract influence nutrient uptake, chlorophyll content and yield. Seaweed extracts showed resistance against fungal attack and sucking insect attack. Several studies have confirmed the favourable outcome of seaweed extract applications on plant growth and yield (Sivasankari *et al.,* 2006a,b,c; Balakrishnan *et al.,* 2007; Kannathasan *et al.,* 2008; Zodape *et al.,* 2010; Kalaivanan and Venkatesalu,. 2012; Johnson *et al.,* 2014; Ganapathy Selvam and Sivakumar, 2016).

The centre of origin of *Arachis hypogaea* is most likely Central Brazil. The cultivated species of *Arachis hypogaea* has been probably originated from a wild allotetraploid origin. *Arachis hypogaea* is a major edible-oil seed crop in India being grown on an area of about 7.92 million hectares with annual production of 8.26 million tons of nut in shell. It is a major crop in most tropical and subtropical area of the world being grown on over 20 million hectares. In the oil seed scenario of India, *Arachis hypogaea* is the largest component which occupies 45% of the total oil seed area and contributes 55% of the production. Although India ranks first in world in terms of both groundnut area and production its productivity is low (1042 kg/ha). About 13.5 million ha are grown in Asia, 5.3 million in Africa, 1.2 million ha in other parts of world. The present study was carried out to understand the efficacy of the brown seaweed *Sargassum polycystum* as a liquid fertilizer on *Arachis hypogaea* var. Co 1.

Vegetative cells are found in many "Glands cells" "Secretory cells" and "Vesicle cells". Because the function of these cells is unknown, the ultrastructure and histochemistry of the vacuole provide evidence that the foamy appearance is a normal developmental phenomenon. The vesicle cells have been described as secretory cells secretion, in the strict sense is the cellular process of synthesizing, segregating and releasing some substances from the cell.

Peanut (*Arachis hypogaea* L.) is an important crop for edible oil and protein, which is grown mainly in semi-arid tropic and sub-tropic areas of 109 countries around the world. A variety of molecular, chemical, and morphological descriptions are used to characterize the genetic diversity among and within crop species. Molecular marker techniques including random amplified polymorphic DNA (RAPD) (Williams and Gwyn, 1991) have been used to study polymorphism in groundnut. Different protocols for DNA extraction have been applied to control and SLF treated leaves which were modified to provide DNA extraction protocol, suitable for several kinds of genetic studies in plants (Doyle and Jeff, 1987), DNA extraction is an important step in molecular assay and plays a vital role in obtaining high resolution results in gel-based systems, particularly in the case of cereals with high content of interfering components in the early steps of DNA extraction. A prerequisite for taking advantage of these methods is the ability to isolate genomic DNA of superior quality and quantity for analyzing through

Polymerase Chain Reaction (PCR), The problem of DNA extraction is still an important issue in the field of plant molecular biology. Various plants contain high levels of polysaccharides and many types of secondary metabolites affecting DNA purification. Certain polysaccharides are known to inhibit RAPD reactions (Pandey *et al.,* 1997).

The application of molecular markers for the estimation of the variability of plant varieties and species is helpful in both detection of genetic relationships between them and making a system of plant genera, which involves the most important agricultural species.

The study on molecular characterization of seaweed liquid fertilizer treated *Arachis hypogaea* using PCR-RAPD markers reported that RAPD analysis is a highly suitable method for the detection of DNA polymorphism in treated peanut. The RAPD marker generated genetic variation among control and SLF treated plant to help the distinguish the plant showing differences in morphological characters.

MATERIALS AND METHODS

Collection of seaweed

The brown alga selected for the present study was *S. polycystum* C. Agardh and it occurs abundantly upto 5 m depth in Nochiyurani coast (09 16.'N' 78 02, 43'E) is located near mandapam coast in the Gulf of Mannar. The intertidal region of Nochiyurani coast is dominated by beach rocks which were formed from lithificaiton by calcium carbonate sediment in the intertidal and spray zone. The hard substratum of the rocks favours for the growth of diverse marine macro algal species.

The collected plants were first washed thoroughly with seawater to remove the salt on their surface and then with distilled water. The washed material was dried in the shade at room temperature. The dried algae was powdered using a mixi. The powder was sieved and preserved in plastic containers. The SLF was prepared from this powder as per the method of Rama Rao (1990).

Selection of crop plant

The crop plant selected for the present study was *Arachis hypogaea* L. because of it's best oil yielding capacity and wild cultivation in India. The certified seeds of *Arachis hypogaea* Var. Co 1 was obtained from Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu. It was well suited in pot culture and later it was transferred to field. The selected seeds were uniform size, colour and weight for experimental purpose were stored in metal tin as suggested by Rao (1976).

Preparation of seaweed liquid fertilizer

The method of Rama Rao (1990) was followed for the preparation of SLF. Twenty five grams of *S. polycystum* fine powder was taken in a conical flask and added distilled water in the ratio of 1:20 (w/v). The mixture was autoclaved at 121°C for 30 minutes, filtered through cheese cloth and collected the filtrate. The filtrate was centrifuged in a cooling centrifuge at 10,000 rpm at 4°C for 30 minutes. After centrifugation, the supernatant collected was used as concentrated SLF. From the supernatant different concentration (Control, 10, 25, 50, 75, 100%) of SLF were prepared using distilled water.

Experimental plantation

For pot culture studies, the pot (1 ½ height; 1 diameter) were filled with sand, soil and farmyard manure in the ratio of 1:2:1. The seeds of *A. hypogaea* were sterilized with 0.012%. Mercury chloride and then washed thrice in distilled water. The sterilized 30 seeds were soaked in each concentrations of SLF for 5 hrs, while control was maintained by soaking the seeds in a beaker containing equal volume of distilled water. After treatments, both control and treated seeds were sown in pots which were maintained in the Botanical Garden of Department of Botany, Annamalai University, the Var. Co 1 *Arachis hypogaea* was well suited in pot culture and was later transferred to the field for experimental studies during the period January 2017 to March 2017. The germination percentage, flowering, pods, fresh and dry weight, growth parameters were measured and biochemical constituents such as chlorophyll 'a' and 'b' (Arnon, 1949), carotenoid (Mackinney, 1941), protein (Lowery *et al.,* 1951) reducing sugar and total sugar (Nelson, 1944) were estimated.

Light microscopic studies

The materials of leaf, stem, root and root nodules were preserved in 70% ethanol. Cross sections of leaf, stem, root and root nodules were prepared by hand from preserved material in chloral hydrate and sartur reagent. Sartur reagent contains KI-I, aniline, Sudan III, lactic acid, alcohol and water. Illustrations were made using a Leitz drawing prism attached to a Leitz-Wetzlar (45º) microscope. Photomicrographs were taken with a camera adapted to Magnum MLX microscope.

Scanning Electron Microscopic studies

Scanning Electron Microscopic studies of groundnut was done by using the method of Hayat (1970). Groundnut (leaf, stem, root and root nodules) were fixed in primary fixative 3% glutaraldehyde. The fixed sample were given 3 washes thoroughly in 0.1 M phosphate buffer (pH 6.8) they were dehydrated through a graded series of alcohol 10-15 minutes interval at 4°C up to 70%. Then 90% and 100% alcohol were kept in room temperature at 2-3 h. interval. Dehydrated samples were treated with critical point drier (CPD) were mounted on a stub and the specimens were examined with Joel JSM-56010 with INSA-EDS and electron micrograph were taken selectively from the computer screen.

Transmission Electron Microscopic studies of leaves

Leaves of *Arachis hypogaea* were cut into 1-2 mm bits and fixed on the spot with 3% glutanaldehyde in 0.1 M phosphate buffer (pH 7), specimen were post-fixed in 1% OSO_4 in the same buffer overnight at 4°C. Then they were washed thoroughly with buffer and dehydrated in an upgraded series of alcohol (Hayat, 1981). Infiltration and embeeeding were performed with Spurr resin. Ultrathin sections of 60-90 nm were taken with a Reichart Jung Ultramicrotome using glass knives. The sections were double stained with Uranyle acetate and lead citrate (Reynolds, 1963) electron micrograph were taken under (CM-Philips Transmission Electron Microscope).

Isolation of genomic DNA

The samples of young and tender leaves of control and SLF treated plants of *Arachis hypogaea* were collected from Botanical garden, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. After washing the plant tissue with sterile water and subsequently with 70 per cent alcohol, 1g of fresh leaf tissue was taken and then it was chopped into fine pieces and subjected to genomic DNA by modified CTAB method.

Modified DNA extraction method of CTAB was as follow

(1) Preheat CTAB buffer in water bath at 65°C. Grind 1g of young leaves to fine powder in ice cold condition in the presence of 100mg PVP (Poly Vinyl Pyrrolidone) by using pre chilled mortar and pestle (-40C/-80C). (2) Transfer the contain in 2 ml micro centrifuge tubes and suspend in two volumes of CTAB buffer. (3) Invert and mix gently and incubate at 60°C for 30 min. (4) centrifuge the suspension at 10,000 rpm for 10 min at room temperature. (5) add 1.5 mL of extraction buffer and incubate at 60°C for 30 min (6) Centrifuge at 10,000 rpm for 10 min at room temperature. (7) carefully transfer the aqueous phase into a new tube. (8) add double volume of chloroform:Isoamyl alcohol (24:1) and invert gently 10 to 15 times and centrifuge at 10,000 rpm for 10 min. (9) Add double volume of chilled isopropanol and keep at -20°C for one hour to precipitate the DNA. (10) Centrifuge at 10,000 rpm for 10 min and discard the supernatant. (11) To the pellet, add 70% chilled ethanol and spool out the pellet carefully and centrifuge again at 10,000 rpm for 10 min. (12) Discard the supernatant and vacuum dry or air dry the pellet at room temperature. (13) Add 100 ml of high salt TE buffer. (14) Add 3uL RNase and keep at 37°C for 30 min. (14) Add 3 M

Sodium acetate (15) Spool out the DNA, wash in 70% ethanol, air or vacuum dry. (16) add 30 to 50 ul (depending upon the pellet) of TE buffer to dissolve the precipitate.(17) store at -20°C/-40°C till further use.

Qualitative and Quantitative analysis of Extracted DNA

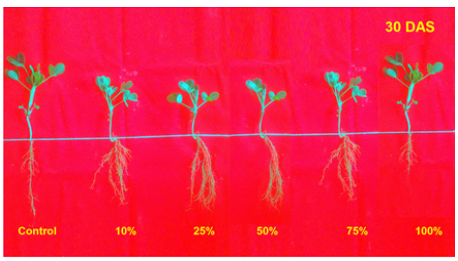
The DNA yield per gram of control and SLF treated leaves tissue extracted was measured using a Nano photometer (Implen, P360 Version 1.2.0) at 260 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260/280 nm. DNA concentration and purity was also determined by running the samples 0.8% agarose gel based on the intensities of band when compared with the lambda DNA marker (Used to determine the concentration).

PCR-RAPD analysis

The PCR amplification reaction was carried out with ten Oligo nucleotide OPA primers from 1 to 10 series obtained from GeNei (Bangalore). Each 20 ul reaction volume containing 10mM TrisHCl (pH 8.3), 2.5mM $Mgcl_2$, 25mM dNTPs mix, 0.2uM of each primer, 10x Taq buffer, 1U of Taq DNA Polymerase and 50 ng of template DNA. RAPD-PCR was performed in Master cycler nexus (Eppendorf) for 40 cycles consisting of denaturation at 94°C for 45 sec, annealing at 38°C for 50 sec, and extension at 72°C for 60 sec. The final extension was carried out at the same temperature for 10 min and the hold temperature of 4°C at the end. The PCR amplified product were electrophoresed on 2% (w/v) agarose gels, in 1x TAE buffer at 65 V for 3 hrs and then stained with ethidium bromide (0.5ug/ml). Gels with amplification fragments were visualized and photographed under UV gel documentation system (Alpha Innotech). Lambda DNA was used as molecular marker (GeNei, Bangalore) to know the size of the fragments.

RESULTS AND DISCUSSION

The effect of different concentrations of *S. polycystum* liquid fertilizer on seed germination and growth parameters of *A. hypogaea* var. CO 1 are presented in Table 1. The highest germination percentage (98.9%) was recorded at 25% concentration. The seed germination percentage increased upto 25% concentration and thereafter it decreased. The lowest germination percentage (58%) was recorded at 100% concentration. The effect of liquid fertilizer *S. polycystum* on fresh and dry weight, flowering in 40 days grown plants of *Arachis hypogaea* is given in Table 3. The maximum fresh and dry weight (27.78,6.89 g/plant) and flowering (23rd day after sowing) were observed in 25% concentration treated plants, while decrease in fresh and dry weight (14.8, 1.80 g/plant) and flowering (30th day after sowing) were observed at 100% concentration treated plants (Figs. 1 and 2).



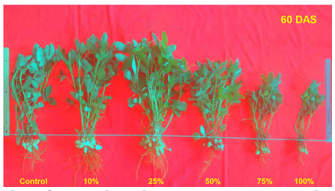


Fig. 1. Growth of Arachis hypogaea treated with different concentration of seaweed liquid fertilizer of S.polycystum on 30and 60 DAS

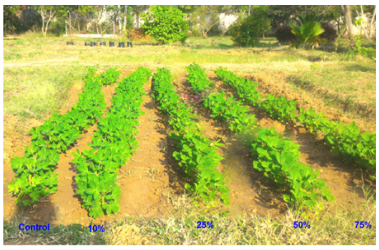


Fig. 2. Growth of *Arachis hypogaea* plant treated with liquid fertilizer(25%) of *S. polycystum*

The seeds of *A. hypogaea* Var. Co 1 treated with low concentration of SLF of *S. polycystum* showed high number of germination whereas the seeds treated with higher concentrations of SLF showed low number of germination. The lower concentrations of SLF enhanced the rate of seed germination and may be due to the presence of growth promoting substances such as IAA and IBA, gibberellins (GA), cytokinins, micronutrients (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins and amino acids (Challen and Hemingway, 1965). The present observations agree with the earlier studies in cereals and vegetables (Dhargalkar and Untawale, 1983), *Cajanus cajan* (Mohan *et al.,* 1994, cereals and millets (Rajkumar Immanuel and Subramanian, 1999), *Beta vulgaris* (Stephenson, 1974), *Vigna catjung* and *Dolichos biflorus* (Anantharaj and Venkatesalu, 2001-2002), *Lycopersicon lycopersicum, Abelmoschus esculentus* (Selvaraj *et al.,* 2004), *Vigna sinensis* (Sivasankari *et al.,* 2006, a,b).

The effect of SLF on growth parameters of *A. hypogaea* studied is presented in Table 1 and 2. The highest root length (13, 14.98 cm/plant), shoot length (19.74, 36.65 cm/plant), number of nodules (40, 92 / plant), internode length (3.24, 3.86 cm/plnat), number of branches (4, 6/plnat), number of leaves (34 plant), leaflet length (4.26, 5.6 cm/plant), leaflet breadth (2.36, 2.88 cm/plant), petiole length (3.82, 5.36 cm/plant) and number of pegs (20/plant) were found at 25% concentration of SLF. The lowest root length (6.21, 10.16 cm/plant), shoot length (10.68, 22.16 cm/plant), number of nodules (9, 24/plant), internode length (1.29, 1.60 cm/plant), number of branches (2, 3/plant), number of leaves (17/plant), leaf let length ((2.9, 3.2 cm/plant), leaflet breadth (1.27, 1.99 cm/plant), petiole length (1.53, 3.42 cm/plant) and number of pegs (5/plant) were observed at 100% concentration of SLF. The effect of SLF on the pod production of *A. hypogaea* is presented in Table 3. The highest number of pods (47/plant) was recorded at 25% concentration, whereas lowest number of pods (22) was recorded at 100% concentration.

Table 1. Effect of seaweed liquid fertilizer of *Sargassum polycystum* on growth parameters of *Arachis hypogaea* (20 days)

Concentration (%)	Germination (%)	Root length (cm/plant)	Shoot length (cm/plant)	No. of nodules/ plant	Internode length (cm/plant)	No. of branches/ plant	Leaflet length (cm/plant)	Leaflet breadth (cm/plant)	Petiole length (cm/plant)
Control	86 ± 2.58	9.03 ± 0.2709	11.05 ± 0.3315	24 ± 0.72	1.35 ± 0.0405	2 ± 0.06	2.83 ± 0.0849	1.66 ± 0.0498	3.10 ± 0.093
10%	96 ± 2.88	12.07 ± 0.3621	18.50 ± 0.555	30 ± 0.9	2.35 ± 0.0705	3 ± 0.09	3.60 ± 0.108	2.20 ± 0.066	3.75 ± 0.1125
25%	98 ± 2.94	13.04 ± 0.3912	19.74 ± 0.5922	40 ± 1.2	3.24 ± 0.0972	4 ± 0.12	4.26 ± 0.1278	2.36 ± 0.0708	3.82 ± 0.1146
50%	78 ± 2.34	6.54 ± 0.1962	13.86 ± 0.4158	23 ± 0.69	1.43 ± 0.0429	3 ± 0.09	3.32 ± 0.0996	1.42 ± 0.0426	2.42 ± 0.0726
75%	61 ± 1.83	6.34 ± 0.1902	12.24 ± 0.3672	11 ± 0.33	1.41 ± 0.0423	2 ± 0.06	3.16 ± 0.0948	1.37 ± 0.0411	2.17 ± 0.0651
100%	58 ± 1.74	6.21 ± 0.1863	10.68 ± 0.3204	9 ±0.27	1.29 ± 0.0387	2 ± 0.06	2.9 ± 0.087	1.27 ± 0.0381	1.53 ± 0.0459

Number of sample (n=s) S.D

Table 2. Effect of seaweed liquid fertilizer of Sargassum polycystum on growth parameters of Arachis hypogaea (40 days)

Concentration (%)	Root length (cm/plant)	Shoot length (cm/plant)	No. of leaves/ plant	No. of nodules/ plant	Internode length (cm/plant)	No. of branches / plant	Leaflet length (cm/plant)	Leaflet breadth (cm/plant)	Petiole length (cm/plant)	No. of pegs/ plant
Control	11.38 ± 0.3414	26 ± 0.78	2.24 ± 0.72	66 ± 1.98	2.52 ± 0.0756	4 ± 0.12	3.7 ± 0.111	2.27 ± 0.0681	4.16 ± 0.1248	8 ± 0.24
10%	13.80 ± 0.414	34.56 ± 1.0368	29 ± 0.87	78 ± 2.34	3.54 ± 0.1062	5 ± 0.15	4.86 ± 0.1458	2.64 ± 0.0792	5.08 ± 0.1524	14 ± 0.42
25%	14.98 ± 0.4494	36.65 ± 1.0995	34 ± 1.02	92 ± 2.76	3.86 ± 0.1158	6 ± 0.18	5.6 ± 0.168	2.88 ± 0.0864	5.87 ± 0.1761	20 ± 0.6
50%	11.42 ± 0.3426	28.54 ± 0.8562	22 ± 0.06	56 ± 1.68	2.78 ± 0.0834	3 ± 0.09	4.28 ± 0.1284	2.29 ± 0.0687	4.46 ± 0.1338	12 ± 0.36
75%	11.20 ± 0.336	24.28 ± 0.7284	19 ± 0.57	32 ± 0.96	2.42 ± 0.0726	3 ± 0.09	3.36 ± 0.1008	2.2 ± 0.066	3.12 ± 0.0936	8 ± 0.24
100%	10.16 ± 0.3048	22.16 ± 0.6648	17 ± 0.51	24 ± 0.72	1.60 ± 0.048	3 ± 0.09	3.2 ± 0.096	1.99 ± 0.0597	2.58 ± 0.0774	5 ± 0.15

Number of sample (n=s) S.D

Table 3. Effect of Sargassum polycystum on growth and yield in Arachis hypogaea 60 days after sowing

Concentration (%)	No. of pods/ plant (60 DAS)	Fresh weight (g/plant)	Dry weight (g/plant)	Days to flowering
Control	28 ± 0.84	20.32 ± 0.6096	4.8 ± 0.144	29 ± 0.87
10%	42 ± 1.26	24.18 ± 0.7254	6.42 ± 0.1926	24 ± 0.72
25%	47 ± 1.41	27.78 ± 0.8334	6.89 ± 0.2067	23 ± 0.69
50%	38 ± 1.14	20.14 ± 0.6042	3.680 ± 0.1104	28 ± 0.84
75%	30 ± 0.9	17.24 ± 0.5172	2.16 ± 0.0648	29 ± 0.87
100%	22 ± 0.66	14.8 ± 0.444	1.80 ± 0.054	30 ± 0.9

The growth of *A. hypogaea* was maximum at low concentration of SLF, whereas the higher concentration of SLF decreased the growth. The increase in seedling growth may be due to the presence of phenyl acetic acid (PAA) and other closely related compounds (P-CH-PAA) in the seaweed liquid fertilizer (Taylor and Wilkinson, 1977), as well as the presence of some growth promoting substance and micro and macronutrients in seaweed liquid fertilizer (Challen and Hemingway, 1965). The present observation agree with the results on *Zea mays* (Stephenson, 1974), *Zizyphus mauratiana* (Rama Rao, 1991), *Cajanus cajan* (Venkatraman Kumar *et al.,* 1993; Mohan *et al.,* 1994), *Abelmoschus esculentus* (Selvaraj *et al.,* 2004) and *Vigna sinensis* (Sivasankari *et al.,* 2006b).

The low concentration of SLF increased the chlorophyll 'a' and 'b' and carotenoid content of *A. hypogaea* while decrease in chlorophyll 'a' and 'b' and carotenoid content were recorded at higher concentration of SLF. The increased chlorophyll 'a' and 'b' and carotenoid in treated with low concentrations of SLF may be due to the presence of nickel at low level in seaweed extract (Narwal *et al.,* 1996), iron, copper and magnesium elements which act as a catalyst for the synthesis and maintenance of chlorophyll (Paul and Norgkynirh, 1996). These findings coincide with the earlier studies in *Phaseolus mungo* and *Cymopsis tetragonoloba* (Lingakumar *et al.,* 2006), *Vigna mungo* (Venkataramakumar and Mohan, 1997) and *Sorghum bicolor* (Thirumal Thangam and Maria Vicotria Rani, 2006).

Data collected on the effect of SLF on biochemical constituents of *A. hypogea* are presented in Tables 4 and 5. Maximum and minimum biochemical constituents were found at 25% and 100% concentration of SLF respectively. The highest chlorophyll 'a' content (1.5182, 2.3459 mg/g fr. wt), chlorophyll 'b' ((0.7598, 1.1058 mg/g fr. wt), carotenoid (0.543, 0.7648 mg/g fr. wt.), protein (2.6428, 3.572 mg/fr. wt.), reducing sugar (5.6368, 8.8184 mg/g fr. wt.) and total sugar (12.4382, 15.9128 mg/g fr. wt.), were recoded at 25% concentration SLF treated plants.

Table 4. Effect of SLF of Sargassum polycystum on Arachis hypogaea biochemical constituents (20 days)

Concentration (%)	Chlorophyll 'a' (mg/g fr. wt.)	Chlorophyll 'b' (mg/g fr. wt.)	Carotenoid (mg/g fr. wt.)	Protein (mg/g fr. wt.)	Reducing sugar (mg/g fr. wt.)	Total sugar (mg/g fr. wt.)
Control	1.2138 ± 0.0364	0.6786 ± 0.0203	0.3834 ± 0.0115	1.7518 ± 0.0525	4.5408 ± 0.1362	8.1929 ± 0.2457
10%	1.4928 ± 0.0447	0.7366 ± 0.0220	0.5125 ± 0.0153	2.5398 ± 0.0761	5.3456 ± 0.1603	10.4896 ± 0.3146
25%	1.5282 ± 0.0458	0.7598 ± 0.0227	0.5432 ± 0.0162	2.6428 ± 0.0792	5.6368 ± 0.1691	12.4382 ± 0.3731
50%	1.1886 ± 0.0356	0.6808 ± 0.0204	0.3864 ± 0.0115	2.1318 ± 0.0639	4.3476 ± 0.1304	8.6416 ± 0.2592
75%	1.0768 ± 0.0323	0.6656 ± 0.0199	0.3619 ± 0.0108	1.7422 ± 0.0522	4.2436 ± 0.1273	8.0608 ± 0.2418
100%	0.9068 ± 0.0272	0.6082 ± 0.0182	0.2854 ± 0.085	1.1630 ± 0.0348	3.5325 ± 0.1059	5.1348 + 0.1540

Number of sample (n=s) S.D

Table 5. Effect of SLF of Sargassum polycystum on pigment and biochemical contents of Arachis hypogaea Var. Co. 1 (DAS 40)

Concentration (%)	Chlorophyll 'a' (mg/g fr. wt.)	Chlorophyll 'b' (mg/g fr. wt.)	Carotenoid (mg/g fr. wt.)	Protein (mg/g fr. wt.)	Reducing sugar (mg/g fr. wt.)	Total sugar (mg/g fr. wt.)
Control	2.1159 ± 0.0634	1.0752 ± 0.0322	0.6966 ± 0.0208	3.3716 ± 0.1011	6.6983 ± 0.2009	12.0817 ± 0.3624
10%	2.2968 ± 0.0689	1.0926 ± 0.0327	0.7397 ± 0.0221	3.464 ± 0.1039	8.1868 ± 0.2456	15.1862 ± 0.4555
25%	2.3459 ± 0.0703	1.1058 ± 0.0331	0.7648 ± 0.0229	3.572 ± 0.1071	8.8184 ± 0.2645	15.9128 ± 0.4773
50%	2.1836 ± 0.0655	1.0624 ± 0.0318	0.6342 ± 0.0190	3.326 ± 0.0997	6.616 ± 0.1984	13.366 ± 0.4009
75%	1.9386 ± 0.0581	0.9432 ± 0.0282	0.4986 ± 0.0149	3.1984 ± 0.0959	6.5246 ± 0.1957	12.0646 ± 0.3619
100%	1.8876 ± 0.0566	0.8262 0.0247	0.3784 ± 0.0113	2.7314 ± 0.0819	5.6439 ± 0.1693	10.0618 0.3018

Number of sample (n=s) ± S.D

The chlorophyll 'a' (0.9068, 1.8876 mg/g fr. wt.), chlorophyll 'b' (0.6082, 0.8262 mg/g fr. wt.), carotenoid (0.2854, 0.384 mg/g fr. wt.), protein (1.1630, 2.7314 mg/g fr. wt.), reducing sugar (3.5328, 5.6439 mg/g fr. wt.) and total sugar (5.1348, 10.0618 mg/g fr. wt.) were found lowest at 100% concentration SLF treated plants.

The lower concentrations of SLF of *S. polycystum* increased protein content in *A. hypogea* whereas higher concentration of SLF decreased protein content was observed. The increased protein content of lower concentrations of SLF of *S. polycystum* might be due to the absorption of most of the necessary elements by the seedling (Kannan and Tamilselvan, 1990) and the presence of various elements in seaweed liquid fertilizer (Selvaraj *et al.,* 2004). Similar results were also reported in *Vigna catjung* and *Dolichos biflorus* (Anantharaj and Venkatesalu, 2001, 2002), *Vigna radiata* (Sivasankari *et al.,* 2006a). The total sugar and reducing sugar were gradually increased upto 25% concentrations of SLF treated plants and thereafter this content gradually decreased upto 100% concentration of SLF. The similar observations were made in earlier studies on *Vigna mungo* (Venkataraman Kumar *et al.,* 1993), *Vigna catjang* and *Dolichos biflorus* (Anantharaj and Venkatesalu, 2001, 2002, Sivasankari *et al.,* 2006b), *Phaseolus mungo* (Lingakumar *et al.,*

2004). The number of pods also increased with lower concentrations of SLF of *S. polycystum,* while the number of pods decreased with increase in the concentration. Similar observations was made in tomato (Rajeswari *et al.,* 1983), sweet corn and tomato (Aitken and Senn, 1965), *Abelmoschus esculentus* and *Lycopersicum lycopersicon* (Selvaraj *et al.,* 2004).

Influence of seaweed extract as an organic fertilizer on the growth and yield of *Arachis hypogaea* L. and their elemental composition using SEM-Energy Dispersive Spectroscopic Analysis was made (Ganapathy Selvam and Sivakumar, 2014). Micromorphological study of *Vigna mungo* using seaweed liquid fertilizer from *Hypnea musciformis* was made (Ganapathy Selvam and Sivakumar, 2016).

Scanning Electron Microscopic Observation

Root

The basic primary structure is typical of leguminous crop plant, with an epidermis formed by small cells, a broad critical zone of parenchyma cells arranged readily in its inner part and alternately in its peripheral region and a broad center cylinder with tetrarch organization of the vascular system (Fig. 3a and b).

Lateral roots originate from the pericycle. Vascular tissues are in radial arrangement. The tissue by which xylem and phloem are separated is called conjunctive tissues. Thickness of xylem is 8-12 mm. Xylem is in extract condition. Each phloem patch consists of sieve tubes, companion cells and phloem parenchyma metaxylem vessels are generally polygonal in shape (Fig. 3b).

Root Nodules

Root nodules occur on the roots of plants that associate with symbiotic nitrogen-fixing bacteria. Under nitrogen-limiting conditions, capable plants form a symbiotic relationship with a host-specific strain of bacteria known as rhizobia.

Nodule inception initially relies on cell competence in a narrow infection zone located just behind the growing root tip. Older nodules then regulate the number of nodules on a root system by suppressing the development of nodule primordial (Fig. 3e).

Stem

SLF 25% treated plant stem, epidermal cell, is a single layer of parenchymatous rectangular cells. The cells are compactly arranged without intercellular spaces. Outer walls of the epidermal cells have a single layer called cuticle. Cross section, measuring thickness of cortex is 10-15 mm. Endodermis is made up of single layer of barrel shaped parenchymatous cells. All the tissues present inside endodermis comprise the stele, pericyte is generally a single layer of parenchymatous cells found inner to the endodermis (Fig. 3c and d).

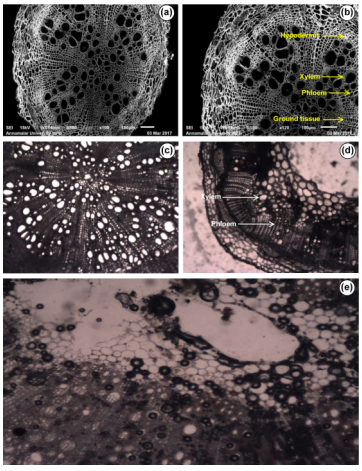


Fig. 3. Scanning electron microscopic image of root, root nodules, stem of A. hypogaea treated with 25% S. polycystum (a) Root, (b) Root a portion enlarged view,(c)Stem, (d) Stem a portion enlarged view, (e) Root nodules

Leaf

The A. hypogaea (SLF) 25% treated plant leaf, the cuticle is thickest layer cross section, measuring cuticle thickness is 10-15 mm. Epidermis is usually made up of a single layer of cells that are closely packed. It is 9-13 mm thickness. Vascular tissues are present in the veins of leaf vascular bundles are conjoint, collated and closed xylem cell individual and width is 8-10 mm, it's thickening size is 3-5 mm (Fig. 4a-e).

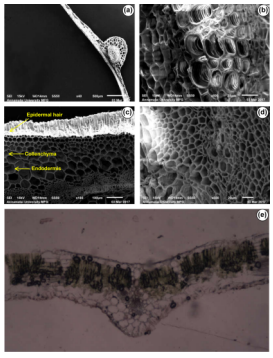


Fig. 4.Scanning electron microscopic image of leaf of *A. hypogaea* treated with 25% *S. polycystum* (a) Leaf, (b)Ultrastructure of leaf shown storage of oil glands (c) Hypodermis layer, (d) Enlarged view, (e) Light microscopic image of leaf epidermal portion of *A. hypogaea* treated with 25% SLF of *S. polycystum*

Scanning Electron Micrograph of *A. hypogaea* control plant in root, stem and leaf section were showed tissues had poorly developed under compared to SLF treated plant (Fig. 5 a-c).

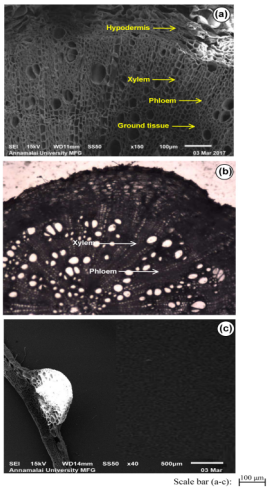


Plate 5. Scanning electron microscopic image of *A. hypogaea* control plant in (a) root, (b) stem and (c) leaf Transmission Electron Microscopic studies of *Arachis hypogaea*

Endomorphological observation of leaf cells in *Arachis hypogaea* was well preserved by primary fixation of glutaraldehyde and post fixation of osmium tetraxide, as shown by TEM, (Fig. 6a-g) the TEM micrograph showed that the cells were formed from the inwardly from the central cell a large inclusion was anchored to the cell wall (Fig. 6a) This inclusion was highly vacuolated containing many electron - opaque matrix (Fig. 6a) some of the vacuoles appeared to be partially osmiophilic, although these more likely represent artificates due to glancing section of the vacuole membrane.

The cores contain less protein and more unknown material than the matrix. Part or all of the vacuolar material is synthesized by abundant rough endoplasmic reticulum (ER) and deposited in smooth surfaced cisternae that swell to form vesicles (Fig. 6b). The vesicles enlarge by continued deposition of synthesized

material and coalescence with other vesicles. All vesicles eventually coalesce to form the mature vacuole. A crystalline array of fibrils develops in the cytoplasm during later stages of vacuole enlargement. Active release of the vacuolar material does not occur, and organelles for extracellular secretion are not present. Structural evidence suggests a storage, rather than secretory, function for the cells (Fig. 6b).

The ultrastructure of a developing vesicle cell shows abundant rough endoplasmic reticulum (RER) the enlarging vacuole is bounded by a single membrane and contains an electron opaque, noncrystalline material consisting of spherical or irregularly shaped zones, or cores of less opacity surrounded by a matrix of greater electron opacity (Fig. 6c). Vesicles appear to be coalescing with each other and the developing vacuole distributed throughout the cytoplasm are mitochondria associated with stacks of smooth surfaced cisternae containing either electron opaque or electron transparent contents. The nuclear envelope is characteristically in complete, with 34-750 nm gaps a prominent nucleolus is usually associated with a gap in the nuclear envelope, Ribosome-like particles are present in the nucleolus in the envelope gaps, free in the cytoplasm and associated with the ER (Fig. 6c & d).

Chloroplasts are infrequent in the vesicle cells as only 19% of the cells contained chloroplasts. When present, the chloroplasts are located in the vicinity of the nucleus and are typical of vegetative cell chloroplasts isolated starch grains were never observed in the vesicle cells. The initial stages of vacuole formation are characterized by the presence of smooth surfaced electron dense vesicles, 0.1-1.0µm, which are disposed throughout the cytoplasm continuities between the smooth ER-like cisternae and the enlarging vesicles suggest that the electron dense material is reduced in the abundant RER and sequestered in the smooth ER (SER) continued deposition of the cisternal material and enlargement of interconnected cisternae result in vesicle coalescence to form the developing vacuole.

More mature vesicles contain a peripheral layer of dense material surrounding less dense zones similar to the vacuolar cores. Continued coalescence of the vesicles results in differential electron densities of the developing and mature vacuole. The vacuole enlarges distally from the vesicle cell branch pressing the cytoplasm into a thin, parietal layer containing RER, mitochondria and dictyosomes. The mature vacuole is entire and surrounded completely by the vacuolar membrane. Vesicle membranes are not present within the vacuole (Fig. 6e & f).

Gland cells are formed soon after the apical division and can occupy a large portion of the volume, up to 10% cellular inclusion ultrastructure studies revealed that, originating from gland cells is comprised of numerous electron translucent vacuoles enclosed by an electron –opaque matrix. Light microscopy further revealed that stalk-like structures connected the gland cell to the outer wall of the pericentral cell. These stalk like structures may provide the mechanism for metabolite transfer to the surface. Gland cell walls are relatively thin, which in turn would aid the transfer of metabolites to the stalk- like structure. These features of the gland cells provide essential clues to the production and storage of the halogenated metabolites in *Arachis hypogaea* (Fig. 6 e & f).

Vegetative cells of *Arachis hypogaea* contain chloroplasts, mitochondria, dictyosomes and protein crystals in the cytoplasm surrounding a central vacuole that contains small, spherical, proteinaceous inclusions. In contrast, the highly specialized vesicle cell is characterized by abundant RER, numerous ER- derived vesicles that form a large vacuole and a cytoplasmic, nonproteinaceous crystal (Fig. 6 g). Chloroplast segregation occurs formation in and a similar process may occur during vesicle cell formation.

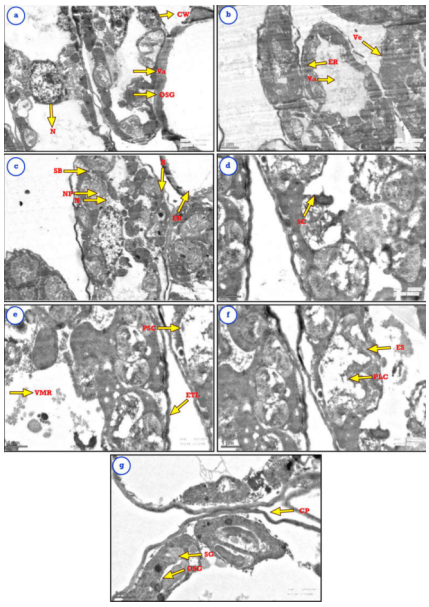


Fig. 6. Transmission electron microscopic

(a) TEM micrograph showed the central cell a large inclusion was anchored to the cell wall and also electron dense and electron translucent material was observed. (Fig. 15a)
CW - Cell wall
V - Vacuole
OSG - Osmiophilic granules
N -Nucleus

(b) The vacuolar material is synthesized by abundant endoplasmic reticulum, vesicles enlarged be continued deposition of synthesized material and coalescence with other vesicles (Fig. 15b).
ER- Endoplasmic reticulum
V- Vesicle

(c) Enlarged vacuole is bound by a single membrane and contains, electron opaque, non crystalline material consisting of spherical or irregularly shaped bounded bodies were observed the nuclear envelop is characteristically in complete a prominent nuclear is usually associated with a gap in the nuclear envelop ribosome like particles present in the nucleolus (Fig. 15c).
R- Ribosomes
SP- Spherical bodies
NP- Nuclear pore

(d) Ultrastructure and chemical portioning of the vacuolar material is the vesicles cells lived the foamy structure of the vesicle cell vacuole to the due to cell senescence these vesicle cells have been described as secretary cells, secretion in the strict sense in the cellular process of synthesizing segregating and releasing some substance from the cell (Fig. 15d).

SC- Secretary cell

(e&f) Protein crystals present in the vegetative cell seems to represents more convenient form of reserve materials in view of the complex ultra structural and biochemical events occurring during vesicle cell development these unlikely that the cells represent excretory structures although phenolic compounds may present in the vacuole (Fig-15 e and f).
VMR- Vacuolar material retamed
ETL- Electron translucent layer
PSC- Protein secreting cell
VGC- Vesiculated gland cell
PC- Protein crystal
ES- Excretory structure
PLC- Phenolic compounds.

(g) Chloroplast degeneration in the vesicle cells or by a failure to

distribute plastids to the vesicle cell initial chloroplast secrecation occurs during mutation formation in *Arachis hypogaea* and a similar process may occur during the vesicle cell formation (Fig. 6g).
CP- Chloroplast
SG- Starch grain
OSG- Osmiophilic globules

RAPD marker analysis in the SLF treated *A. hypogaea*

This marker analysis in the SLF treated *A. hypogaea*. The study revealed the DNA polymorphism through RAPD-PCR reaction in *A. hypogaea*. In this reaction consists of disappearance of normal bands and appearance of new bands obtained and compared to both treated and untreated plants (Tables 6 - 8).

RAPD – PCR and it showed (Fig. 7) that 10 primers could reveal some polymorphism in the amplified in Fig. 7 DNA sample ranged from 100 to 1000 bp in size. All the ten amplified primers were examined for DNA polymorphism. Out of 10 primers used 8 primers produced amplification where as 2 primers OPA-9 and OPA-10 did not show any amplification. The polymorphism ranged from 25 to 100%. Maximum numbers of monomorphic , polymorphic and rare bands were observed in OPA-6 and OPA-8. Similar results have already been reported by Skroch and Nienhuis (1995) that RAPD bands amplified by one primer vary in intensity from those amplified by another primer. Earlier, random amplification of polymorphic DNA analysis was conducted on the genetic resources of *Plantago* sp. To access genetic variability (Modgil *et al.,* 2005). His conclusion confirmed to a wide variation of phenotype in the cultivated peanut, which contradicted with some previous studies (Kochert *et al.,* 1991). Generally, natural plant tissue are not preferred for DNA extraction due to mainly to the presence of high concentration of polysaccharides, polyphenols and other secondary metabolites (Doyle and Chef, 1987; Pandey *et al.,* 1997) referred to as the OD260/280 ratio.

The mean OD260/280 nm ratios from modified CTAB method were higher than 1.9 in these three methods RNA disposal was not involved, hence there existed some RNA residues, as determined by the electrophoresis on Agarose gel (Fig. 8), there were clear main bands observed).

The amplification of *Arachis hypogaea* DNA was observed only on the OPA-1 to OPA-8 series of primers. There is no amplification for OPA-9 and OPA-10th series of primers. As the observed DNA extracted from the selected CTAB Modified method, had good amplification and also had the good banding pattern (Fig. 9).

Table 6. Different Concentration Of Modified Ctab Method

Name of the Solutions	CTAB - Standard Concentration Method	CTAB - Modified Concentration Method
EDTA	20 mM	50 mM
Tris –HCl	100 mM	120 mM
NaCl	1.5 M	1M
B-Mercapto ethanol	2%	1.5%

Table 7. Comparison Of Quality And Quantity Of Genomic Dna Isolated From Control And Slf Treated Leaves Of Arachis Hypogaea

Methods	Control leaves		SLF Treated Leaves	
	DNA yield (ug/g)	A260/A280	DNA yield (ug/g)	A260/A280
Commercial Kit	1.54±0.04	1.57±0.04	1.94±0.05	1.97±0.05
Standard CTAB	17.72±1.94	1.64±0.07	22.12±1.85	1.74±0.07
CTAB Modified Method	62.61±1.93	1.84±0.05	88.11±2.81	1.92±0.05

The results are mean of triplicates determination ± standard deviation. Data are means ± SD (n=3).

Table 8. Details Of Primers Using RAPD-PCR Analysis On Dna From Arachis Hypogaea By Using CTAB Modified Method

S. No.	Primer	Sequences
1	OPA-01	5'-CAGGCCCTTC -3'
2	OPA-02	5'-TGCCGAGCTG -3'
3	OPA-03	5'-AGTCAGCCAC -3'
4	OPA-04	5'-AATCGGGCTG -3'
5	OPA-05	5'-AGGGGTCTTG -3'
6	OPA-06	5'-GGTCCCTGAC -3'
7	OPA-07	5'-GAAACGGGTG -3'
8	OPA-08	5'-GTGACGTAGG -3'
9	OPA-09	5'-GGGTAACGCC -3'
10	OPA-10	5'-GTGATCGCAG -3'

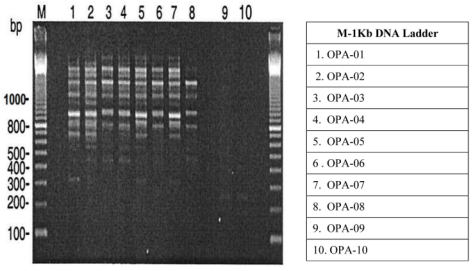


Fig. 7. Polymerase chain reaction using ten primers on DNA from leaves samples of Arachis hypogaea by using CTAB modified method

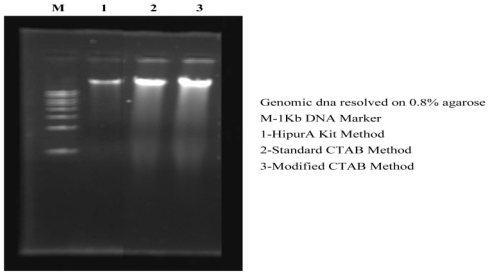


Fig. 8. Genomic DNA of Arachis hypogaea resolved in 0.8% of Agarose Gel Electrophoresis

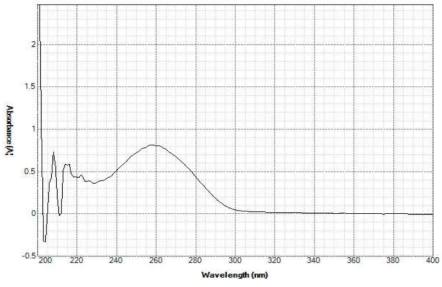


Fig. 9. Absorption spectrum of DNA isolated from SLF treated leaves of *Arachis hypogaea* by using modified CTAB method

CONCLUSION

In agriculture, seaweeds are applied as soil conditioner, fertilizer and green manure due to the presence of high amount of potassium salts, micronutrients and growth substances chiefly cytokinin. The SLF prepared from marine macro alga *S. polycystum* was found to be superior in promoting seed germination, growth, yield and biochemical constituents in *Arachis hypogaea* Var. Co 1. Hence, this type of application of eco-friendly SLF to crop plants is recommended for the farmers to get higher yield and products. Seaweed liquid fertilizer treated plants gave reproducible with wide variations in their band numbers at the molecule level. Both SLF treated and RAPD marker are important for genetic analysis and confirmation a great approach of genetic analysis between the different studies of *Arachis hypogaea*.

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