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Morphological parameters and carbohydrate accumulation of rice cultivars as influenced by seaweed extract application under aerobic conditions

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ABSTRACT

The present study was conducted to investigate the effect of seaweed extract (SWE)on morphological indices and carbohydrate accumulation of two rice cultivars under aerobic conditions. The experiment was laid out in factorial completely randomized design (CRD) with three replications. SWE @ 10.0%, 12.5% and 15.0% w/v were applied as soil drench at 3 DAS. The results revealed that SWE application showed positive effect on plant vigour, biomass production and total leaf area in both rice cultivars. SWE application showed negative effect on number of leaves in cultivar PR 116. Relative growth rate and vigour index was recorded higher in cultivar PR 116 as compared to Nagina 22. The increase in total soluble sugar and starch content was also observed in both cultivars with SWE application. It was concluded that SWE had a promontory effect on the morphological attributes and carbohydrate accumulation under aerobic conditions but effect was found to be more pronounced in cultivar PR 116.

Key words: *SWE, carbohydrate, biomass, leaf area and starch*

Rice is the most important cereal crop for more than three billion people, over half of the world's population (Phung, 2014**)**. It was grown in more than a hundred countries with total cultivated area of about 160 million hectares and occupied 11% of the world's cultivated area with the production of more than 700 million tons. Rice production at regional and national level was adversely affected by irrigation difficulties, uncertainties of rainfall, limited irrigation facilities, land and water resources towards traditional rice cultivation method (Sangeetha, 2014**)**. Hence use of resource conservation technology is inevitable in rice based cropping system (Jadhav *et al.*, 2014**).** Aerobic rice is a water-saving rice production system in which potentially high yielding, fertilizer responsive, highly adapted rice varieties were grown in well-drained, non-puddled, non flooded and non-saturated aerobic soil without ponded water (Jana *et al.,* 2015). Soil water at saturated condition or above did not affect water productivity, light related parameters, soil chemical properties and plant production (Khairi *et al.,* 2015**).** It was estimated that the world population would increase 9 - 11 billion by the year 2025 out of which 4.3 billion would be dependent on rice for their basic food (Bisne *et al.*, 2009). To meet the food requirement of the growing population, farmers were using chemical fertilizers to enhance crop production. Modern agriculture is searching for new strategies that would allow optimize the use of chemical inputs to sustain crop productivity (Satapathy *et al.,* 2014). In recent years, SWE was used as an alternative of conventional synthetic fertilizers to increase plant growth and yield, and improve negative cropping conditions such as the progressive degradation of ecosystems and the contamination of agricultural lands. In India, as a step towards the expansion of native sources of natural manures, the seaweed fertilizers application would be useful now to sustain plant production. These were marketed as biostimulants or biofertilizers due to presence of a wide variety of plant growth-promoting substances such as auxins, cytokinins and betaines. Kumari *et al* (2011) reported that aqueous extracts of *Sargassum johnstonii* @ 0.1 to 0.8% (w/v) enhanced vegetative growth and reproductive parameters of tomato seedlings. The aim of this experiment was to study the effect of SWE on morphological indices and carbohydrate content of rice cultivars under aerobic conditions.

MATERIALS AND METHODS

The present investigation was conducted in lab of Department of Botany, Punjab Agricultural University, Ludhiana. It is situated at 30^0-54^0 N latitude, 75^0-45'E longitude and at a mean height of 247 meters above sea level. It is placed in south-Central plain region of Punjab having subtropical and semi-arid climate.

The seeds of two rice cvs PR 116 and Nagina 22 were procured from the Director Seed, PAU Ludhiana, Punjab. These were surface sterilized with 0.1 % mercuric chloride for 2-3 min to avoid any fungal infection. Twenty seeds of each cultivar were sown in pots and placed in incubator with a temperature of 30 ± 2^0C. After three days, application of SWE @ 10%, 12.5 % and 15 % (w/v) as soil drench was given. The treatments were allotted in completely randomized design (CRD) in three replications. The following treatments were included:

T1 - Control (no SWE application)
T2 -T1 + SWE @ 10.0%
T3 -T1 + SWE @ 12.5%
T4 -T1 + SWE @ 15.0%

Randomly five plants were selected in each pot at 14 DAS for recording observations on plant growth parameters (fresh weight, dry weight, total leaf area and leaf number), physiological parameters (vigour index and relative growth rate) and the biochemical parameters (total soluble sugars and starch content).

The seedling fresh weight was recorded in milligrams with the help of balance. These seedlings were dried in oven at 60^0C until constant weight was obtained. Dry weight was recorded in milligrams by using balance.

The number of leaves was counted and their average was recorded. The leaf area was calculated by formula given by Bhan and Pande (1966)

Leaf area = Length x Breadth x 0.75 (constant for rice)
Total leaf area = Leaf area of all leaves

Length and breadth of leaves were measured in cm by using centrimetre scale.

Vigour index of seeds was calculated as Germination (%) x Epicotyl length (cm)

Epicotyl length of seedlings was measured by using centimetre scale.

Relative Growth Rate (RGR)

$$RGR = \frac{Dw}{t} \times \frac{1}{W_0}$$

W_0 = Initial dry weight (mg)
D_W = Change in dry weight (mg) in time 't'
t = Time interval (in days)

Total soluble sugars were estimated by method as given by Dubios *et al* (1956). The total soluble sugars were extracted from dried leaves using 80% ethyl alcohol. Absorbance was taken at 490 nm in spectrophotometer. The quantity of sugars was calculated against the standard curve prepared by using pure glucose (10-100µg) and expressed as mg g^{-1} dry weight.

The residue left after sugar extraction was used for starch estimation (Maryada 2006). The residue was washed 4-5 times with 80% ethanol to remove all traces of soluble sugars. The absorbance was read at 630 nm. The quantity of sugars was calculated against the standard curve prepared by using pure glucose (10-100µg). The quantity of sugar was multiplied with 0.9 factor to estimate starch content and expressed as mg g^{-1} dry weight.

The data on morphological and biochemical parameters were evaluated and analysis of variance (ANOVA) was done statistically using CPCS1 software. The effect of SWE concentration and cultivars were evaluated by the least significant difference (LSD) test at $P<0.05$.

RESULTS AND DISCUSSION

In the present study, the application of seaweed extract enhanced plant growth parameters such as biomass production, total leaf area, leaf number, plant vigour and relative growth rate. Such enhancement in growth parameters could be attributed to either improvement in soil condition resulting in greater root growth thereby enhanced the utilization of soil nutrients or the changes in the biotic and abiotic environment of plants.

Plant biomass was associated with dry matter production and it depended on leaf number and leaf area. It was also affected by the concentration of the nutrients supplied as well as quantity of photosynthetic products of the plant. In comparison among the cultivars, the maximum plant biomass (fresh and dry weight) was recorded in PR 116 (93.50 and 22.16) as compared to Nagina 22 (89.50 and 20.50) under control conditions (Table 1). The interaction of treatments and cultivars showed non significant differences for fresh weight. Application of SWE at different concentration enhanced fresh and dry weight in both cultivars although the increase was more pronounced in the PR 116 cultivar. SWE at higher concentration (15%) was found to be better than lower concentrations (10% and 12.5%) in enhancing plant biomass. The increased biomass might be due to the presence of higher levels of N, P, K in the seaweed extract. Similar trend was recorded by Kalaivanan *et al* (2012) in which application of SLE of *C. scalpelliformis* increased fresh and dry weight of *Vigna mungo* seedlings. The increase in fresh (SFW) and dry weight (SDW) of beans seedling with SWE application was also observed by Salma *et al* (2014).

In present study, the both cutivars had showed different behavior for number of leaves (Table 1). The non significant differences in leaf number were observed in both the cultivars. Results showed that SWE application at all concentration showed inhibitory effect on number of leaves in PR 116 as compared to control, while in Nagina 22, application of SWE at lower concentrations viz. 10% and 12.5% had significantly increased leaf number and higher concentration viz. 15% showed no stimulatory effect on number of leaves. The ameliorating role of SWE might be due to the high level of cytokinins, auxins, growth hormones and other nutrients. These results were supported by Sutharsan *et al* (2014) in which foliar application of seaweed extract of *Sargassum crassifolium* at lower concentration favoured average leaves number in tomato, while higher concentration exhibited inhibitory effect on leaves number. Rayorath *et al* (2008) also reported that sea weed extract of *A nodosum* enhanced number of leaves of Arabidopsis at very low concentration.

Earlier studies reported that increase in leaf area led to increase in dry matter accumulation because proportion of dry matter allocated to leaves remain fairly constant, while an increase in leaf area led to an increase in rate of dry matter accumulation because light interception was directly related to leaf area during this phase of development. The results revealed that the responses of total leaf area to SWE treatment were similar to those observed with plant biomass, though differences were small and variability was high (Table 1). Among studied cultivars, PR 116 had more total leaf area than that of the Nagina 22 under control conditions. The application of SWE resulted in increase in total leaf area in both cultivars but the effect was found to be more pronounced in cultivar PR 116. The maximum leaf area (3.87) was recorded in rice cultivar PR 116 when SWE was applied at the higher concentration viz. 15% as compared to low concentrations (10% and 12.5%). This phenomenon

Table 1. Influence of SWE application on fresh and dry weight, total leaf area and number of leaves in PR 116 and Nagina 22 cvs. of rice under aerobic conditions

CultivarsTreatments	Fresh weight (mgs)		Dry weight (mgs)		Total leaf area (cm^2)		Number of leaves	
	PR 116	Nagina 22	PR 116	Nagina 22	PR 116	Nagina 22	PR 116	Nagina 22
AC (control)	93.50	89.50	22.16	20.50	3.15	2.47	2.20	2.00
SWE @ 10%	96.00	92.50	25.00	22.00	3.28	2.90	2.00	2.50
SWE @ 12.5%	99.80	93.60	28.75	23.50	3.53	3.11	2.00	2.50
SWE @ 15%	101.40	96.40	30.80	25.00	3.87	3.54	2.00	2.00
CD ($P < 0.05$)	T=1.2227, C=0.8646, TxC= NS		T=1.2234, C =0.8650, TxC=1.7302		T=NS,C =NS,TxC =NS		T=NS,C =NS,TxC =NS	

might be due to presence of macronutrients in SWE which stimulated plant growth as a result of enhancement of protein synthesis, cell division and mobilization of nutrients needed for growth. These results are in conformity with the reports of Sasikumar *et al* (2011),who conducted an experiment of foliar application of seaweed (*Dictyota dichotoma*) on *Abelmoschus esculantus* and the same results were also obtained by Thirumaran *et al* (2009).The same trend was recorded in almonds by Saa *et al* (2015).

There was a direct relationship between relative growth rate and dry matter accumulation or biomass production. The present investigation revealed that RGR varied significantly among studied cultivars (Table 2). The highest RGR was recorded in PR 116 (0.201) followed by Nagina 22 (0.190) under control conditions. The application of SWE showed positive and stimulatory effect on relative growth rate in both cultivars but the effect was found to be more pronounced when higher concentration of SWE viz 15% was applied as compared to lower concentrations. Same results were obtained by Sutharsan *et al* (2014) in which application of *Sargassum crassifolium* extract enhanced root dry matter accumulation in tomato seedlings.

The vigour index was found to be directly related to germination rate and epicotyl or hypocotyl length. Among studied cultivars, PR 116 possesed high vigour index (322.00) as compared to Nagina 22 (305.25) under control conditions. Application of SWE at different concentration showed positive influence on vigour index in both cultivars as SWE induced leakage of inhibitors possibly abscissic acid from the seeds and improved germination rate (Table 2). The maximum vigour index (370.50) was recorded in PR 116 followed by Nagina 22 (360.00) when higher concentration of SWE was applied as compared to lower concentration. The beneficial effect of the seaweeds extracts on germination and growth of various land plants might be due to the presence of plant growth-promoting substances/hormones in the extracts (Thorsen *et al* 2010; Prasad *et al* 2010, Blunden *et al* 2010). Several studies examined the effect of seaweed extracts on seed germination of various plants such as table beet, lettuce, tomato, green gram and black gram (Demir *et al* 2006, Kalaivanan and Venkatesalu 2012, Ashok-Kumar *et al.*, 2012, Ganapathy *et al.*, 2013).

In present study, the sugar and starch content values varied non significantly among studied cultivars (Table 2). The maximum carbohydrate accumulation (5.99 and 2.01) was recorded in rice cultivar PR 116 as compared to Nagina 22 (5.96 and 1.98) under control conditions. The results revealed that application of SWE in all the concentrations resulted in increase in sugar and starch content although the effect was more pronounced in cultivar PR 116 followed by Nagina 22. The maximum sugar (6.17) and starch concentration (2.13) was recorded in PR 116 with SWE application at higher concentration (15%). Such a rise in total sugar and starch content might attributed to better availability of necessary nutrients, minerals (Ca, Na, K, Mg, N and Zn), water and better growth of root with SWE application. Increase in α and β amylase activities due to the presence of growth promoting substance like gibberellins in SWE also enhanced sugar and starch content. Earlier studies reported that application of SWE incresaed reducing and total sugar content of *Vigna mungo* seedlings (Kalaivanan *et al.*, 2012). The same

Table 2. Effect of SWE on vigour index, relative growth rate and carbohydrate accumulation in PR 116 and Nagina 22 cvs. of rice under aerobic conditions

CultivarsTreatments	Vigour index		Relative growth rate		Sugar content		Starch content	
	PR 116	Nagina 22	PR 116	Nagina 22	PR 116	Nagina 22	PR 116	Nagina 22
AC (control)	322.00	305.25	0.201	0.190	5.99	5.96	2.00	1.98
SWE @ 10%	337.50	325.00	0.211	0.200	6.03	6.00	2.04	2.01
SWE @ 12.5%	360.00	346.00	0.216	0.213	6.10	6.07	2.09	2.05
SWE @ 15%	370.50	360.00	0.228	0.221	6.17	6.12	2.13	2.11
CD (P<0.05)	T=1.2329, C=0.8717, TxC= 1.7435		T=0.1219, C=0.8622, TxC=0.1724		T=NS, C=NS, TxC=NS		T=NS, C=NS, TxC=NS	

T=Treatments, C= Cultivars

trend was recorded in *Vigna sinensis*, *Cajanus cajan*, *Lycopersicon esculentum*, *Brassica nigra* and *Cyamopsis teragonolaba* (Sivasankari *et al.,* 2006, Sivasankari Ramya *et al.,* 2011 Kumari *et al.,* 2011, Erulan *et al.,* 2009 and Kalidass *et al.,* 2010).

The presence of growth regulators and inorganic minerals in sea weed extract made them an excellent choice as organic fertilizer so it could be used as biostimulants directly or mixed with chemical fertilizers to enhance plant growth. In our study, the higher concentration of SWE showed better results for seed germination, biomass production, plant vigour and carbohydrate accumulation than lower ones. Therefore, in places where inorganic fertilizers or water availibility were limited, sea weed extract might provide a powerful and eco-friendly approach to nutrient management and the practice of applying SWE could be recommended to growers to attain better germination and rice production under aerobic conditions.

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