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Influence of Fertilizer Regimes and Water Depths on Clonal Growth, Phenology and Chlorophyll Content of *Scirpus grossus* in Paddy Soil

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ABSTRACT

Scirpus grossus L. is a principal rhizomatous weed in the rice fields, drainage and irrigation canals, river banks, abandoned rice fields and wasteland in Malaysia. This study describes the spatio-temporal growth patterns of aerial plant on fertilized and unfertilized paddy soils. The fertilizer application resulted in more robust aerial plant growth of *S. grossus* with gross population of 97.08 ramets m⁻² compared with 83.67 ramets m⁻² in unfertilized plots 24 weeks after planting of the mother plant. Mean ramets mortality was significantly higher in unfertilized plots than the fertilized plots. Flowering set in earlier among ramets in fertilized paddy soils with 49.56 ramets m⁻² vis-a-vis the unfertilized soil registering ca. 47.79 ramets m⁻², 24 weeks after transplanting of the mother plant. The results showed that *S. grossus* started to flower at weeks 8 under 5 cm and 9th week under 0 cm and highest inflorescence number were recorded at 5 cm water depth. Fertilizer applications did not register any significant difference in mean plant height, chlorophyll content, chlorophyll fluorescence measurements and inflorescence production vis-a-vis those devoid of fertilizer application. However, there was a clear reduction in the chlorophyll content when the plants were grown under 20 cm of water in the presence of fertilizers. The time-and space-mediated clonal growth of *S. grossus* did not register any significant preferential directionality and dispersion of aerial plants irrespective of fertilizer regimes and water depths but rather displaying opportunistic resource capture by aerial and sub-terrestrial modules.

Key words: Fertilizer regimes, water depths, clonal growth, phenology, *Scirpus grossus*

INTRODUCTION

Rhizomatous plants grow and reproduce clonally by rhizomes. Clonal branches are formed from the reiteration of the basic units while inflorescence and inflorescences come from the reiteration of units bearing modified leaves (Horn, 1978). The process of new growth is often subjected to different pressures, including the change in soil nutrients and resource capture ability among individual plants and their modules. Remobilization of internal nutrient helps to support new growth and is a key mechanism to explain the improved performance of nutrient-loaded plants (Saliful *et al.*, 2008). For example NPK fertilizer has shown to be effective for growth in several *Senecio* sp. *S. madagascariensis* actually has increased competitive advantage over oats

with increasing nitrogen and phosphorus levels (Sindel and Michael, 1992). The sawdust mulch and NPK 20:10:10 fertilizer affect rates on weed flora composition and growth in plantain and was more abundant in mulched plots while the gramminaceous species (19%) were mostly found in bare plots (Hol, 2011). Many researchers have shown that micro-nutrients have a promising effect on the growth and development of crop plants and the use of micronutrients can improve the quality and quantity of agricultural produce (Rafique *et al.*, 2006).

The impact of water level on wetland macrophyte communities, particularly emergent and submerged species, are well documented in the literature (Casanova and Brock, 2000; Maltchik *et al.*, 2007). Similar effects have been reported for amphibious species as well (Casanova and Brock, 2000; Maltchik *et al.*, 2007). Casanova and Brock (2000) reported the deepest depth in their study was 60 cm, on the influence of water depth on macrophyte establishment. They also, reported differences in *Myriophyllum aquaticum* total shoot length, shoot biomass, root biomass and total biomass, over a limited range of water levels. *M. aquaticum* is capable of growing in deeper water depths, however the direct effects of deeper water levels on growth characteristics are still unknown (Hussner *et al.*, 2009). Another study investigated the comparative effects of water level variations on growth characteristics in *M. aquaticum* to determine its growth response, particularly of biomass and plant length and its individual structures under increasing water depths (Wersal and Madsen, 2011). Similarly, the biomass, plant height, crown diameter, flower number and days of blooming of *Begonia xelator* under the effect of different watering frequencies and fertilizer amounts were studied (Sun and Zhang, 2011). The results of main factor analysis indicated the effect of fertilizer amount was greater than that of watering frequency and the value of watering frequency and fertilizer amount matching the optimal indexes was determined as well. The effects of water content and fertilizer on the growth index and quality indexes of *B. xelator* was obtained by single factor analysis (Sun and Zhang, 2011). The same results were obtained with regard to the biomass of *Lactuca sativa* L. under the effects of NPK fertilizer and water content (Sun and Zhang, 2011). In this study, the effects of water depth and fertilizer on clonal growth, phenology and chlorophyll content of *Scirpus grossus* in paddy soil were investigated.

MATERIALS AND METHODS

Paddy soil of Jawa series taken from Tanjung Karang of been placed in the lower part of pots at depth of 20 cm. While the top part for water depth with 4 levels of water depth (D1 = 0 cm water depth (control); D2 = 5 cm; D3 = 10 cm; D4 = 20 cm) by making some holes at the required level. In the same experiment different fertilizer concentrations were applied. Four levels of NPK fertilizer concentrations (F2, F3, F4, F5), were used with F0 as control and F2 = 50 g/500 mL; F3 = 75 g/750 mL, F4 = 100 g/1000 mL, F5 = 125 g/1250 mL. Each young ramet of *S. grossus* was planted in the center of the pots measuring 20×40 cm in size, on paddy soil type from the MARDI Station of Tanjung Karang. A set of 3 replicates were allocated (R1, R2, R3), encompassing 60 plots in a Randomized Complete Block Design (RCBD) (Fig. 1). The relative rates of increase or recruitment for each appropriate parameter were calculated using the equation:

$$R = \log_e N_2 - \log_e N_1 / (t_2 - t_1)$$

where, R is relative rate of increase or recruitment of the values (N_1 , N_2) of each parameter at t_1 and t_2 , respectively.

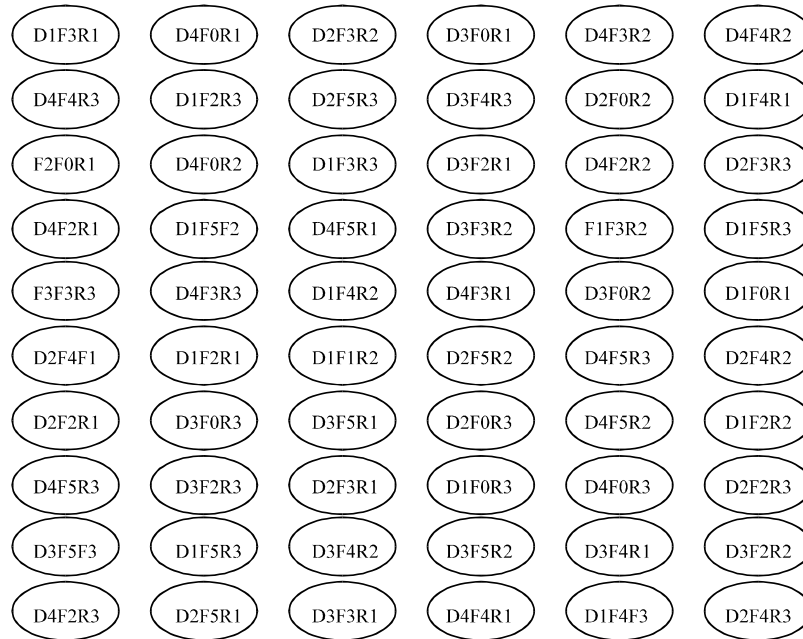


Fig. 1: Experimental design and treatment combinations of pots with different water depths and different concentrations of fertilizer application on *Scirpus grossus* D1 (control) (0 cm), D2 (5 cm), D3 (15 cm), D4 (20 cm); F0 (control) (without fertilizer), F2 (50 g/500 mL), F3 (75 g/750 mL), F4 (100 g/1000 mL), F5 (125 g/1250 mL) and R: Replicates

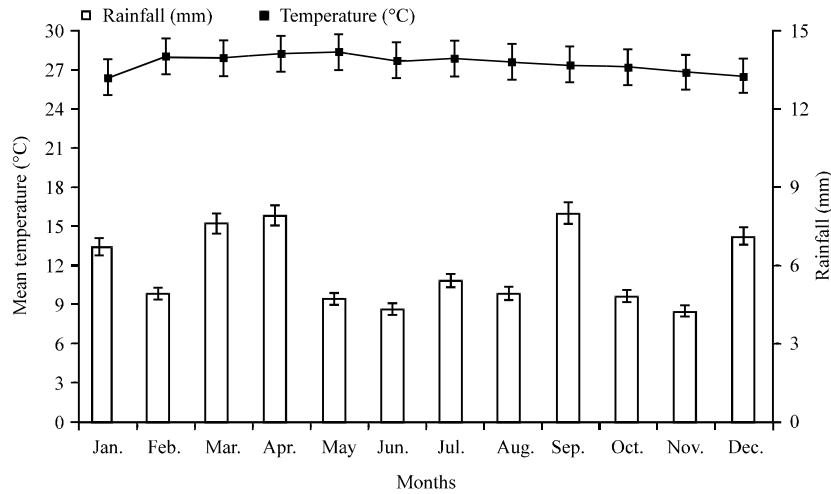


Fig. 2: Mean monthly rainfall (mm) and temperature (°C) readings (2010-2012) in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia. Bars represent highest and lowest rainfall and temperature readings

The weather data on rainfall and ambient temperatures at the experimental sites are shown in Fig. 2 where it can be seen the rainfall ranged from 3.9-10.8 mm and the temperature ranged from 26.5-28.6°C (Meteorology Department Malaysia).

The weather data for Universiti Putra Malaysia is shown in Fig. 2. The inflorescence number were count on every week and chlorophyll content of leaf were measured with a SPAD meter on each 4th week of plant growth.

Data acquisition and management: For measurement of clonal growth pattern of *S. grossus*, each ramet was planted in the canter of a plot measuring 2×2 m, previously demarcated and lined with 5×5 cm grids and sub-plots. The clonal growth of *S. grossus* based on the number of emerged plants in each plots, its position within the 5×5 cm grids and their plant heights were recorded on weekly basis for 24 weeks.

Likewise for the number of dead plants and their positions in each plot within the 5×5 cm grids were also recorded. In the case of the chlorophyll fluorescence which has been well documented to be closely related to photosynthetic capacity and quantum yield was recorded using a Hansatech (UK) Photosynthetic Efficiency Analyzer. Variable (Fv) and maximum fluorescence (Fm) readings were taken and the Fv/Fm values which correspond to quantum efficiency, were calculated. A minimum of five readings per plot for each treatment were taken and the average determined.

After 24 weeks of experimentation, the plants were harvested by dismembering them into leaf, stem and inflorescence components and their dry weights were determined.

For determining the effects of water depth and fertilizer on the inflorescence number and chlorophyll content of leaves, we used a pot experiment with different water depths and different concentrations of fertilizer (Fig. 1). The phenology of *S. grossus* was also recorded, taking into account the time of first flowering after planting and the number flowers were recorded on a weekly basis. The chlorophyll content in the leaves was determined at the end of planting using a Minolta SPAD meter. From each pot 5 leaves were randomly selected for measurement.

Statistical analysis: The data of population fluxes of ramets (number of plant and mortality number) and height plants were transformed to \log^{+1} or \log prior to statistical analysis and subjected to one-way ANOVA and Tukey's HSD tests wherever appropriate, using the SAS Computer Programs. Further analyses were made to determine the significance of planting in fertilized soil and unfertilized soils, planting in paddy soils and weekly differences in general growth pattern and the rate of increase or recruitment of any parameter, e.g., flower number and chlorophyll content by regressing the recruitment values of the \log^{+1} or \log of transformed data against time.

RESULTS

Effect of fertilizer on general growth pattern of *S. grossus*: It has been reported earlier that *Scirpus grossus* plant reiterates by rhizomatous growth and branches from a single mother plant. The results show that the total average gross number of emerged ramets in fertilized soils was higher compared to unfertilized soils at the end of the 24 weeks of study period. The mortality rate recorded was 8.58 ramets in unfertilized soils and 5.67 ramets in fertilized soils (Table 1) while the real rate showed 75.09 ramets m^{-2} in unfertilized soils and 91.41 ramets m^{-2} in fertilized soils. It was also observed that net plant number was also highest in fertilized paddy soil than the unfertilized paddy soil. The highest average plant height in unfertilized soils was 172.67 cm while in fertilized soils it was 175.33 cm. With regard to chlorophyll fluorescence which is indicative of the tissue's photosynthetic capacity, the Fv/Fm ratios were very similar between the two set of plants (Table 1).

Table 1: General growth patterns of *Scirpus grossus* in unfertilized and fertilized paddy soils after 24 weeks of growth

Growth parameters	Unfertilized soil	Fertilized soil
Gross plant number (m ²)	83.67±95.75	97.08±58.25
Mortality number (m ²)	8.58±2.250	5.67±2.250
Net plant number (m ²)	75.09±93.5	91.41±56.00
Plant height (cm)	172.67±4900	175.33±38.50
Chlorophyll fluorescence	0.793±0.019	0.799±0.028
Total leaves weight (g)	10.89±5.25	12.19±5.730
Total stems weight (g)	17.56±3.78	18.61±4.690
Total flowers weight (g)	2.38±0.56	3.13±0.750

Mean±SD of data was significantly different by ANOVA (p<0.001)

The dry biomass of selected plant parts of *S. grossus* taken after harvest at 24 weeks after transplanting displaying measurable differences according to fertilizer regimes are represented in Table 1. The results showed that in unfertilized soils: the leaves were 12.72 g and the stems, 17.56 g whilst the flowers were 2.38 g in weight. In fertilized soils these were measurably higher with 14.84 g (leaves), 18.61 g (stems) and 3.13 g for flowers.

Effect of fertilizer and water depth on inflorescence number: The inflorescences in plants kept in water depths of 10 and 20 cm appeared on the 6th week of experimentation. *Scirpus grossus* started to flower at weeks 8 under 5 cm and 9th week under 0 cm. The results after 16 weeks of study are shown in Fig. 3a. The highest numbers for inflorescence production were recorded at 5, 10, 20 and 0 cm water depths, respectively, where the number of inflorescence observed were as follows; (0 cm = 15.00 m⁻², 5 cm = 25.67 m⁻², 10 cm = 25.00 m⁻², 20 cm = 22.33 m⁻²), respectively. All the results were significantly different at p<0.01 (HSD tests).

Plants started to produce inflorescence in the F2, F3, F4, F5 treatments on the 9th and 10th week while inflorescence appeared earlier in the F0 treatment on the 6th week (Fig. 3b-e). After 16 weeks of study the highest inflorescence number were recorded as follows: F0, F2, F3, F4, F5, respectively. The average inflorescence number for each NPK treatment were as follows; F0 (15.00, 25.67, 25.00, 22.33 m⁻²), followed by F2 (15.33, 14.67, 31.33, 4.33 m⁻²), F3 (14.00, 10.00, 7.00, 1.67 m⁻²), F4 (4.67, 13.00, 12.67, 0.33 m⁻²) and F5 (3.67, 8.33, 9.33, 0.00 m⁻²), respectively. The results were significantly different at p<0.01 (HSD tests). Fertilizer applications did not appear to enhance inflorescence production in *S. grossus* compared with the control.

Effect of water depth and different concentration of fertilizer on leaf chlorophyll: The results in Fig. 4 show that the chlorophyll content was generally higher in plants grown in fertilized soil. The addition of fertilizers increased the chlorophyll content slightly but the increase was not significant. However there was a clear reduction in the chlorophyll content when the plants were grown under 20 cm of water in the presence of fertilizers. At a water depth of 20 cm, increasing fertilizer amounts led to a large drop in chlorophyll content, after only four weeks, with SPAD readings averaging 18.3 and subsequently leading eventually to death after about 12 weeks with SPAD readings dropping to 2.0 (Fig. 4). In the plants that were not treated with fertilizers, the chlorophyll content was not significantly different when grown under different water depths as shown earlier (Fig. 4a-e). A summary of the results with regard to chlorophyll content are

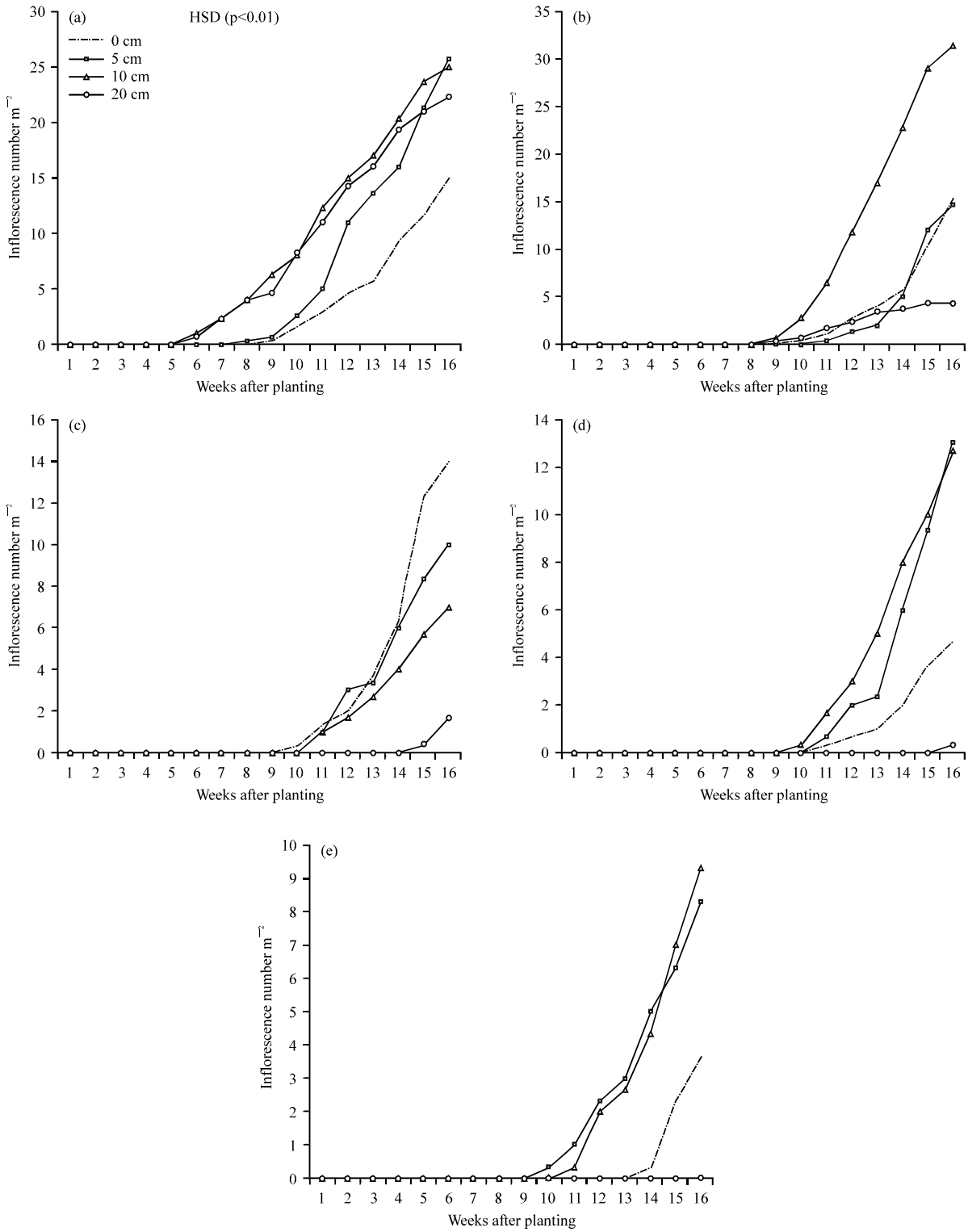


Fig. 3(a-e): Mean inflorescence number of *Scirpus grossus* under different water depths (0, 5, 10 and 20 cm), (a) Without addition of NPK, (b) With 50 g/500 mL (c) With 75 g/750 mL (d) With 100 g/1000 mL NPK and (e) With 125 g/1250 mL NPK application in paddy soil in Universiti Putra Malaysia, Serdang, Selangor, Malaysia

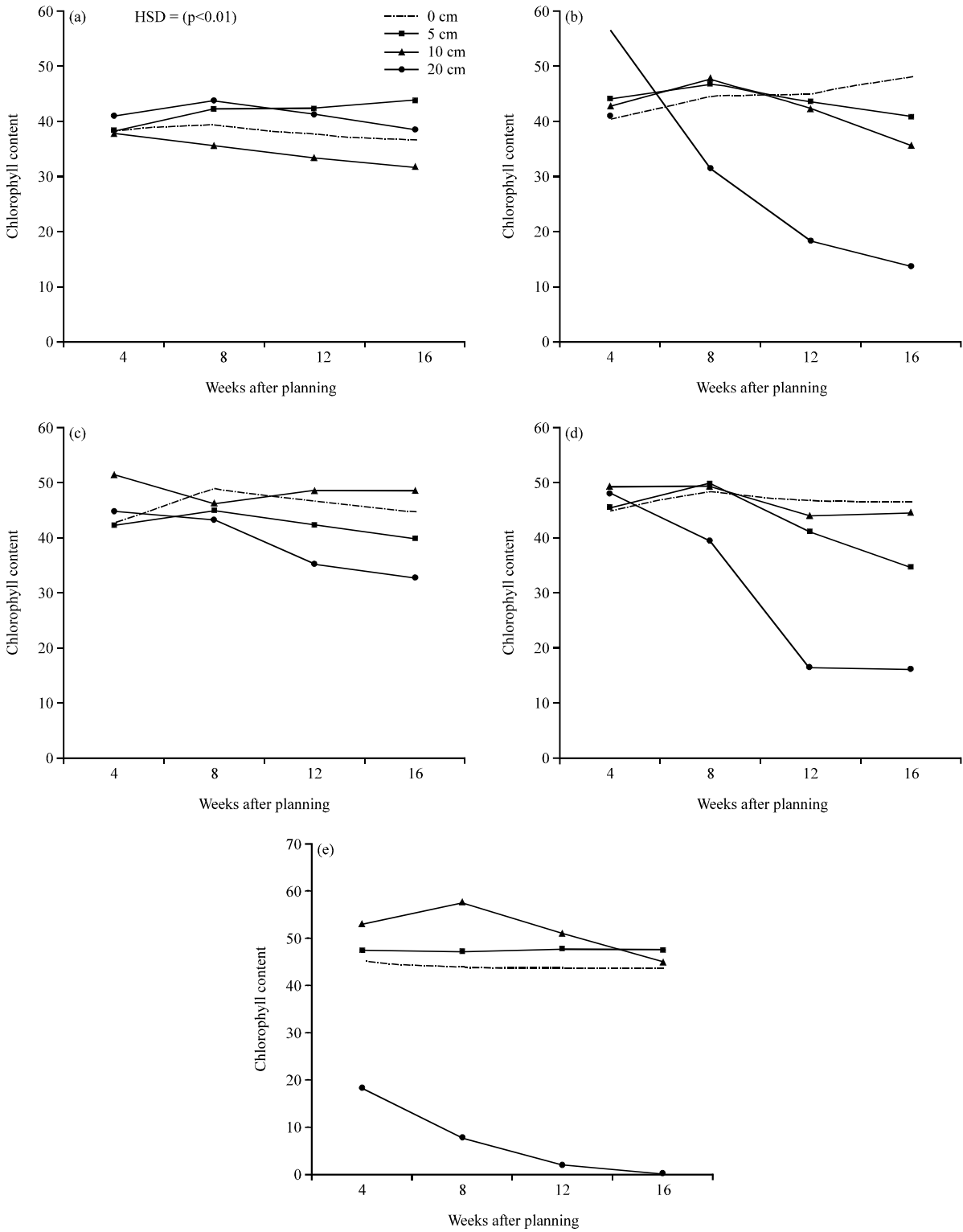


Fig. 4(a-e): Chlorophyll content in *Scirpus grossus* grown under different water depths (0, 5, 10 and 20 cm), (a) Without NPK application, (b) With 50 g/500 mL (c) With 75 g/750 mL, (d) With 100 g/1000 mL and (e) With 125 g/1250 mL NPK application in paddy soil in Universiti Putra Malaysia, Serdang, Selangor, Malaysia

as follows; F0 (0 cm = 36.90, 5 cm = 43.77, 10 cm = 31.73, 20 cm = 38.43), respectively, F2 (0 cm = 48.07, 5 cm = 40.83, 10 cm = 35.60, 20 cm = 13.63), F3X (0 cm = 44.87, 5 cm = 40.00, 10 cm = 48.60, 20 cm = 32.67), F4 (0 cm = 46.57, 5 cm = 34.77, 10 cm = 44.60, 20 cm = 16.03) and at F5 (0 cm = 43.77, 5 cm = 47.67, 10 cm = 45.20, 20 cm = 0.00), respectively. All the results were significant at 0.04% under the influence of two factors together, fertilizer concentration and water depth.

DISCUSSION

This study was an attempt to determine the effect of fertilizer resumes and water depth on the clonal growth, phenology and chlorophyll content of *S. grossus* L. on paddy soil. It showed details of different growth patterns of *S. grossus*. The best period of clonal growth in general was between 10-18 weeks, an outcome similar to the results mentioned by Baki (1988). This phenomenon is probably due to the finite amount of resource in the soil, diminishing with time. The results of this current study showed that the use of fertilizers had a significant impact on content in growth parameter but others did not show a significant impact. The addition of fertilizer had a significant effect on clonal growth where it dramatically increased the population flux of the weed. Similarly, the fertilizer caused a decrease in the number of deaths of ramets and this was similar to the findings of Baki (1988) who studied the structural demography and growth patterns of *S. grossus*. The NPK affected the physiological health of the plant and increased the size of the leaves. In another study phosphate played a significant role in affecting stages of plant growth (Rouached *et al.*, 2011).

In this study, fertilizer treatment had minimal effect on the leaf chlorophyll content and chlorophyll fluorescence, both of which can be interpreted to mean that the plant's photosynthetic capacity was not significantly affected. The Fm/Fv values observed were slightly lower than that documented for healthy leaves which is normally in the region of ~0.0.82 (Murchie *et al.*, 2009). This probably indicates that the soil on which the rice plants were grown had sufficient macro and micro nutrients to support healthy growth during the period of experimentation.

The pattern of reduced flowering were observed in *Typha latifolia* and *T. domingensis* by Grace (1990), who cited carbon budgetary allocation, inducing more clonal growth for continued survivorship rather than allocating more energy for flower production under continued inundation. Since plant response to prolonged sub-mergence includes alterations in architecture, metabolism and elongation growth associated with a low O₂ escape strategy and an antithetical quiescence scheme that allows endurance of continued flooding (Voeselek and Bailey-Serres, 2013), it would not be insensible to argue that *S. grossus* would response likewise perhaps accompanied with a reduction of cellular O₂ content, mediated through multifaceted alterations in cellular and organ structure that promote access to and diffusion of O₂. These processes are driven by phytohormones, including ethylene, gibberellin and abscisic acid. Interestingly, flooding appeared to have enhanced flowering in the sedge. It would be interesting to explore how flooding would change the hormonal budgets in *S. grossus* favouring more clonal growth against the production of inflorescence as shown in this experiment.

The results show chlorophyll content in the leaves of *S. grossus* was subjected to different water depth levels for 16 weeks. As reported above, it was observed that increasing the level of water depth had significant effect on the content of chlorophyll in the leaves of *S. grossus*. However,

Osborne *et al.* (2002) reported that in *Zea mays* L., there was an inverse relationship between water depth and chlorophyll content, with chlorophyll content decreasing with increasing water depth. The negative effects of water on photosynthesis has been reported previously by Tripathy *et al.* (1981) and Panda and Sharma (2007).

This probably indicates that *S. grossus* grows optimally only in a soil in soil inundated with water, such as the paddy soil. Baki (1988) had earlier reported that the growth of *S. grossus* was affected by the depth of inundation with water. Wersal and Madsen (2011) reported significant decline in biomass and plant length as water levels increased, suggesting that submersed leaves alone cannot sustain *M. aquaticum* growth for long periods of time. Earlier, Salvucci and Bowes (1982) reported, optimal photosynthetic rates of *M. aquaticum* were observed in the emergent form and *M. aquaticum* cannot remain as a submersed plant for long periods of time as the photosynthetic rate of submersed leaves may not be sufficient to support plant growth.

Previous studies have reported that leaf photosynthetic rates are reduced in plants grown under water probably providing some evidence for an energetic cost involved in heterophyllous plants (Cook and Johnson, 1968). However, in this study it was observed that there was a decrease in chlorophyll content in the leaves of *S. grossus* when increasing fertilizer concentrations were added to plants grown under 20 cm water depth. This could be due to several reasons, as has been reported in many studies previously, such as the low rates of photosynthesis observed in *M. aquaticum* as the leaves are flooded for extended periods (Salvucci and Bowes, 1982). They suggested that the rate of photosynthesis of the immersed leaves was probably not sufficient to support plant growth. It was also suggested that the growth of the submersed leaves was transient and only utilised for short overwintering periods and times of reduced light and temperature (Sytsma and Anderson, 1993). Another study reported that the presence of algae can lead to reduced hydrocarbon content and works as a contraceptive light and thus inhibiting the growth of *Botryococcus braunii* (Deng *et al.*, 2012). This was observed (high algae growth) in pots submerged in 20 cm water with high fertilizer concentration.

As mentioned earlier, in this study it was observed that water depth did not affect the chlorophyll content. However, when NPK fertilizer was added to plants grown under a depth of 20 cm, a decrease in chlorophyll content was observed, with a greater decrease in increasing fertilizer concentrations and subsequently to the death of the plant. The latter observation could be due to several reasons such as the plant is unable to grow normally under a depth of 20 cm when the fertilizer added becomes toxic as the chemical elements in solution becomes directly readily available to the plant tissues and their high concentration in the tissues will ultimately lead to tissue death.

CONCLUSION

The results of the current study confirm that the fertilizer resumes and water depth did have a significant impact, albeit small, on clonal growth and phenology of *S. grossus*. In addition, the results showed that 5 cm water depth enhance early flowering and increased the inflorescence number. The study also represents a strong evidence that the depth of inundation in excess of 15 cm is deleterious to the chlorophyll content of *S. grossus*. The use of fertilizer resumes and the water depths perhaps are also useful in such an endeavour to further understand behavioural patterns of clonal growth, phenology and chlorophyll content in heterogeneous environments.

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