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Aerial Ramet Dynamics and General Clonal Growth Patterns of *Scirpus grossus* in Grown at Different Water Depths and Fertilizer Regimes in Paddy Soils

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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 provides evidences that plants of *S. grossus* have a remarkable ability to alter their development in response water depth regimes. This phenotypic plasticity allows them to continually adapt to their local environment, a necessity for plants as sessile organisms. The results of this current study showed that devoid of fertilizer application (F0) and not inundated, *S. grossus* plants with mean monthly population counts up to 16 weeks, taken at 4-weekly intervals were as follows: 61.33, 67.33, 75.33 and 117.00 plants m^{-2}. With fertilizer applications, the parallel counts for F2 (64.67, 71.00, 80.33 and 34.33 plants m^{-2}), F3 (65.33, 72.67, 68.33 and 32.00 plants m^{-2}), F4 (71.67, 72.00, 71.67, 6.33 plants m^{-2}), F5 (79.33, 74.67, 52.00 and 0.00 plants m^{-2}), denoting increased mortality of ramets with increased fertilizer applications. Ramet mortality of *S. grossus* increased with the greater depths of the water level and with increasing NPK concentrations. It was also observed that population number of ramets decreased with increased depths of inundation, irrespective of fertilizer regimes. In relation to plant height, recorded results showed that plant height was highest in the 10 cm water depth treatment followed by 20, 5 and 0 cm water depth treatments, respectively. It can be concluded that depth of inundation and fertilizer regimes have significant effects on aerial growth and ramets mortality of *Scirpus grossus*.

Key words: Clonal growth, fertilizer regime, phenology, water depth, plant, soil

INTRODUCTION

Scirpus grossus grows in moist soils or under inundated or water-logged conditions. It is these conditions that may impact on the growth performance of this sedge, particularly when subjected to wet and dry soil fluxes or when subjected to different depths of inundation under the natural environment in drainage and irrigation canals, abandoned rice fields, or even as a weed in rice crops. 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 population dynamics of many rhizomatous plants is dominated more by the flux of clonal modules. The ability of a single genotype to form fragmented phenotypes is just one of the variants in the life patterns of a modular organism (Alderman *et al*., 2011). 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).

It has been well documented in the literature that nitrogen, potassium and phosphorous are important macro elements for healthy plant growth, in addition to other macro-and microelements (Daughtry *et al*., 2000). Nitrogen has been shown to increase the strength of competitive interactions in plants and were far better predictors than growth in the field (Besaw *et al*., 2011). The NPK fertilizer applications usually exhibit superior survival, growth and competitive ability over non-loaded cohorts when transplanted in a variety of habitats (Oliet *et al*., 2009). It has been reported that NPK fertilizer levels had a significant effect on weed population dynamics in onion bulb crop (Patel *et al*., 2011). Another study conducted for 8 years, during which the use of several different tests to improve the quality of NPK fertilizer was made, the results showed that the fertilizer application resulted in lower mortality rate and longer survival of plants (Oliveira *et al*., 2011). The effect of tillage and fertilizer types on soil properties increased significantly leaf area, vine length and tuber yield (39%) in sweet potato (*Ipomoea batatas*) (Agbede, 2010). 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 wet land macrophyte communities, particularly emergent and submerged species, are well documented in the literature (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. *Myriophyllum 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 it is 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 xelatior* under the effect of different watering frequencies and fertilizer amounts was 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. xelatior* 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 (Xu *et al*., 2011). In this study the effects of different concentrations of NPK, different water depths and soil types on the growth patterns of *S. grossus* and important weed in rice fields and waterways, was investigated.

MATERIALS AND METHODS

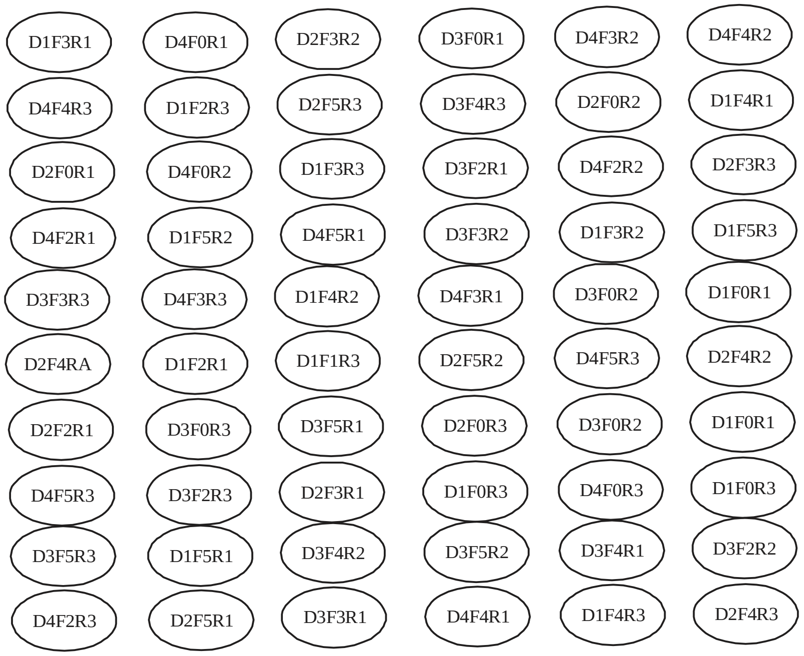
Paddy soil of Jawa series taken from Tanjung Karang of been placed in the lower part of pots at depth of 20 cm. While, the top part for water depth with 4 levels of water depth (D1 = 0 cm water depth (control), D2 = 5 cm, D3 = 10 cm, D4 = 20 cm) by making some holes at the required level. In the same experiment different fertilizer concentrations were applied. Four levels of NPK fertilizer concentrations (F2, F3, F4 and 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 and 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:

$$R = \frac{\mathrm{Log}_e\ N_2 - \log_e\ N_1}{t_2 - t_1}$$

where, R= relative rate of increase or recruitment of the values (N_1, N_2) of each parameter at t_1 and t_2, respectively. 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 26.5-28.6ºC (Meteorology Department Malaysia). The weather data for Universiti Putra Malaysia is shown in Fig. 2.

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 different soils (peat and paddy soils) and weekly differences in the rate of increase or recruitment of any parameter, e.g., leaf number, rhizome length, ramet number, mortality number, plant height, flower number by regressing the recruitment values of the $\log^{+1}$ or log of transformed data against time.



D1F3R1	D4F0R1	D2F3R2	D3F0R1	D4F3R2	D4F4R2
D4F4R3	D1F2R3	D2F5R3	D3F4R3	D2F0R2	D1F4R1
D2F0R1	D4F0R2	D1F3R3	D3F2R1	D4F2R2	D2F3R3
D4F2R1	D1F5R2	D4F5R1	D3F3R2	D1F3R2	D1F5R3
D3F3R3	D4F3R3	D1F4R2	D4F3R1	D3F0R2	D1F0R1
D2F4RA	D1F2R1	D1F1R3	D2F5R2	D4F5R3	D2F4R2
D2F2R1	D3F0R3	D3F5R1	D2F0R3	D3F0R2	D1F0R1
D4F5R3	D3F2R3	D2F3R1	D1F0R3	D4F0R3	D1F0R3
D3F5R3	D1F5R1	D3F4R2	D3F5R2	D3F4R1	D3F2R2
D4F2R3	D2F5R1	D3F3R1	D4F4R1	D1F4R3	D2F4R3

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), R, replicates

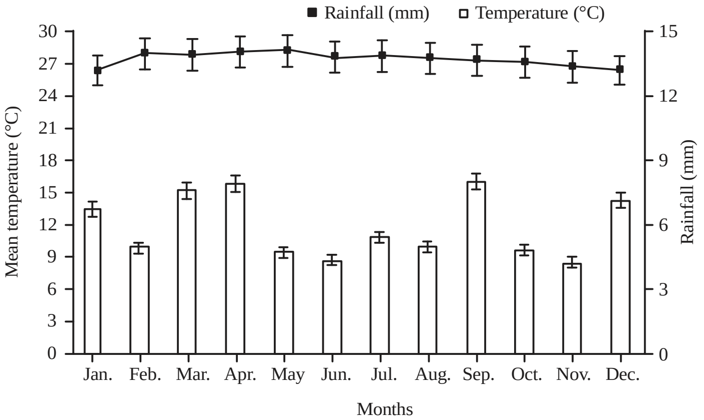


Fig. 2: Mean monthly readings (2010-2012) in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia. Bars represent highest and lowest rainfall and temperature readings

RESULTS

Effects of water depth and fertilizers on number of plant: Table 1 and Fig. 3a showed the clonal growth of *S. grossus* continued until 16 weeks for all the treatments at 0, 5, 10 and 20 cm water depths, taken at monthly intervals. As can be seen in Fig. 3, the results illustrated the effect of water depth level on clonal growth, where plant number increased with increasing water depths. After 16 weeks of clonal growth the readings were as follows, (0 cm depth = 61.33, 5 cm depth = 67.33, 10 cm depth = 75.33, 20 cm depth = 117.00), respectively. All results were significantly different at $p<0.01$ (HSD tests). A similar study with similar results, on the growth and biomass of *Myriophyllum aquaticum* was reported by Wersal and Madsen (2011). It has also been reported

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	83.670±95.75 m^{-2}	97.080±58.25 m^{-2}
Mortality number	8.580±2.25 m^{-2}	5.670±2.25 m^{-2}
Net plant number	75.090±93.5 m^{-2}	91.410±56.00 m^{-2}
Plant height (cm)	172.670±49 cm	175.330±38.5
Chlorophyll fluorescence	0.793±0.019	0.799±0.028
Total leaves weight (g)	10.890±5.25	12.190±5.73
Total stems weight (g)	17.560±3.78	18.610±4.69
Total flowers weight (g)	2.380±0.56	3.130±0.75

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

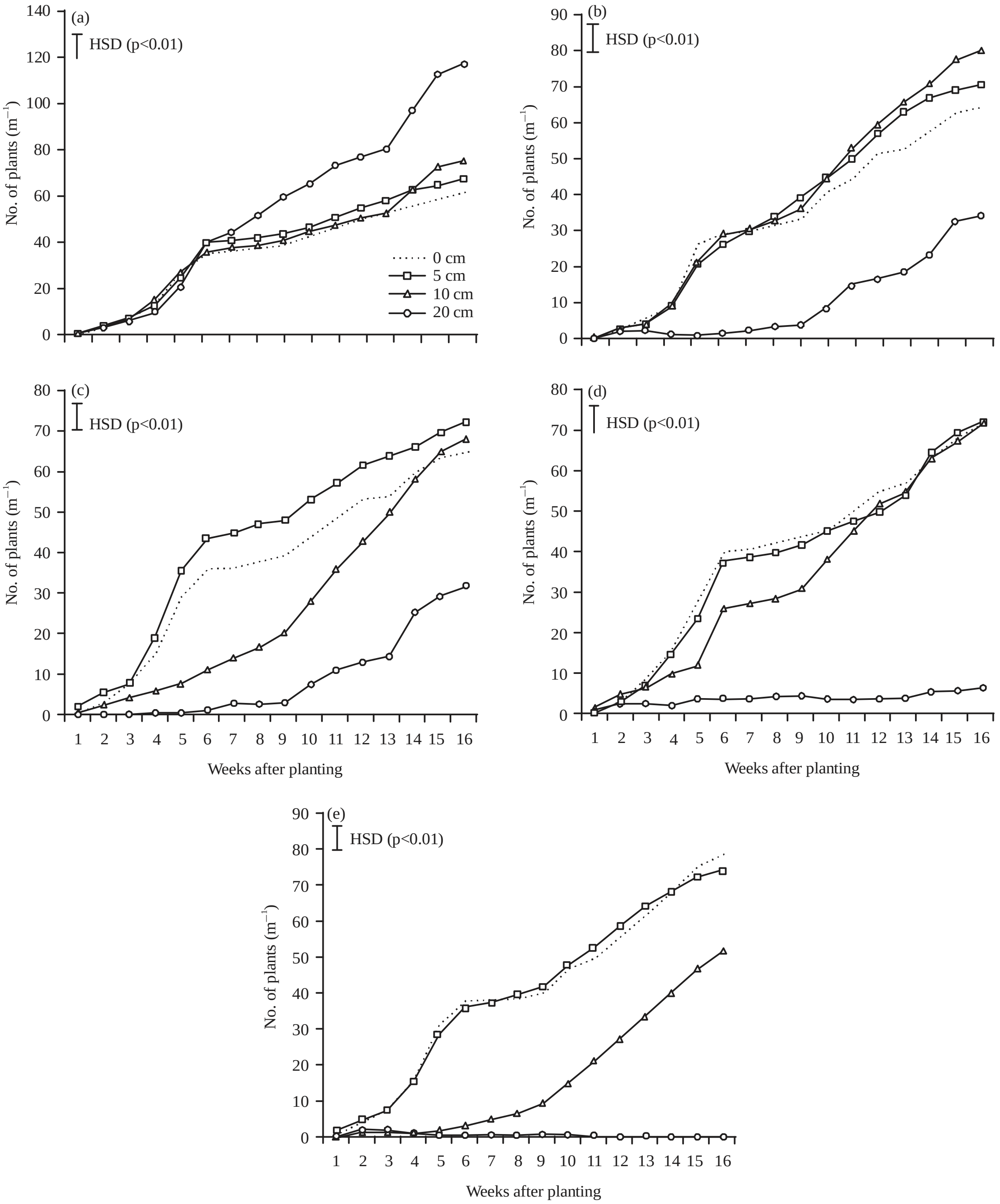


Fig. 3(a-e): Plant number of *Scirpus grossus* under different water depths (0, 5, 10 and 20 cm), (a) Without NPK, (b) 50 g/500 mL NPK, (c) 75 g/750 mL NPK, (d) 100 g/1000 mL NPK and (e) 125 g/1250 mL NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia

previously that biomass allocation to emergent shoots was greater when *M. aquaticum* was grown in water depths of less than 0.5 m (Sytsma and Anderson, 1993).

As shown in Table 1 and Fig. 3b-e the results showed that between the different NPK concentrations used, the highest plant number that emerged was after 16 weeks in all the F0, F2, F3, F4 and F5 treatments. The results also showed that plant number and growth rate showed an increasing trend with increasing NPK concentrations, at water depths of 5 and 10 cm. The monthly population counts with regard to mean plant number up to 4 months, were as follows: F0 (61.33, 67.33, 75.33 and 117.00 plants m^{-2}), F2 (64.67, 71.00, 80.33 and 34.33 plants m^{-2}), F3 (65.33, 72.67, 68.33 and 32.00 plants m^{-2}), F4 (71.67, 72.00, 71.67, 6.33 plants m^{-2}) and F5 (79.33, 74.67, 52.00 and 0.00 plants m^{-2}). It should be noted that for plants growing under water depths of 20 cm, the

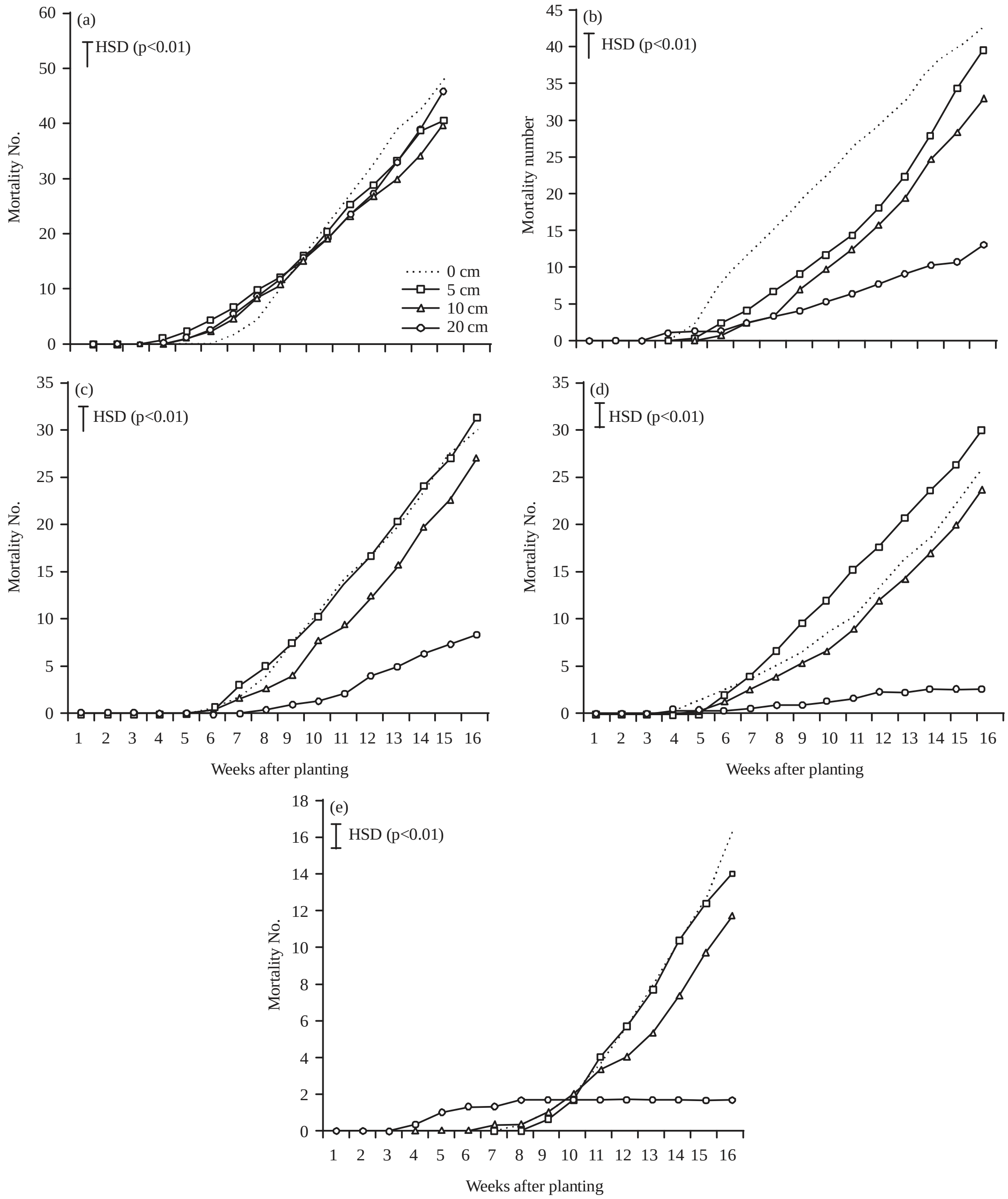


Fig. 4(a-e): Mortality number of *Scirpus grossus* under different water depths (0, 5, 10 and 20 cm), (a) Without addition of NPK50, (b) g/ 500 mL NPK, (c) 75 g/ 750 mL NPK, (d) 100 g/1000 mL NPK and (e) 125 g/1250 mL NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia

F2, F3, F4 and F5 fertilizer concentrations, caused a marked reduction in mean plants m^{-2}. The results were significantly different at $p<0.01$ (HSD tests).

Irrespective of fertilizer application, a host of environmental cues can be interpreted by *S. grossus* ramets including light, temperature and nutrients and these inputs are integrated and translated into a range of developmental outputs from shoot elongation, regulation of root gravitropism, altered flowering time, growth cessation of leaves and bud breaks. In the case of increased water depths, the population increase of ramets were severely curtailed with parallel increase in mortality (Table 1, Fig. 3).

Effects of water depth and fertilizer on ramet mortality: Ramet mortality of *S. grossus* decreased with the greater depths of the water level. The results are shown in Table 1 and Fig. 4a-e, after 16 weeks of ramets mortality readings were as follows; 0 cm depth = 48.33 plants m^{-2}, 5 cm depth = 40.67

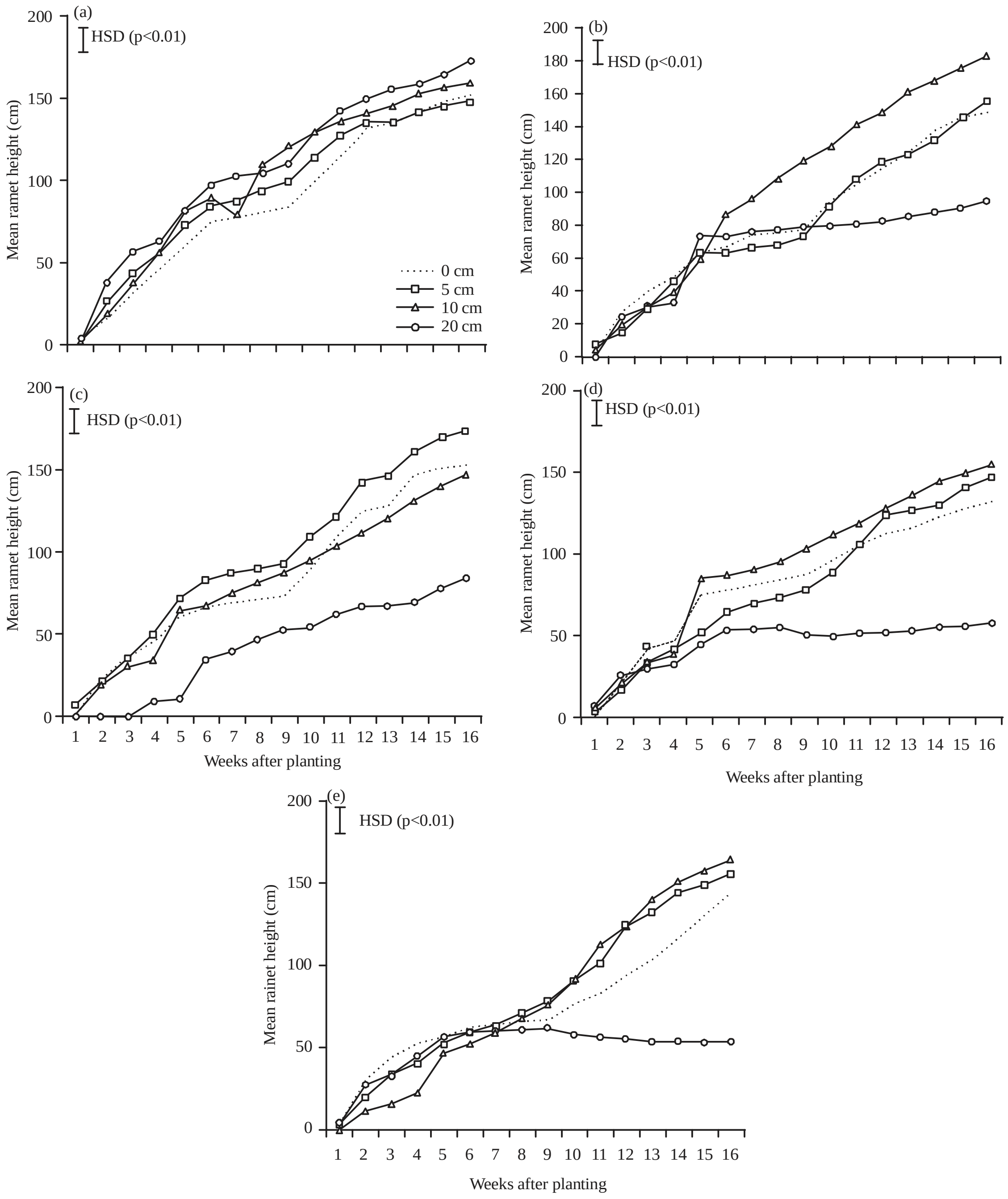


Fig. 5(a-e): Mean ramet (plant) height of *Scirpus grossus* under different water depths (0, 5, 10 and 20 cm), (a) Without NPK, (b) 50 g/500 mL NPK, (c) 75 g/750 mL NPK (d) 100 g/1000 mL NPK and (e) 125 g/1250 mL NPK application in paddy soil in Unversiti Putra Malaysia, Serdang, Selangor, Malaysia

plants m^{-2}, 10 cm depth = 40.00 plants m^{-2}, 20 cm depth = 46.00 plants m^{-2}, respectively. All results were significantly different at p<0.01 (HSD tests).

Effects of water depths and fertilizer regimes on plant height: As shown in Table 1 and Fig. 5a-e, the results showed that between the different NPK concentrations used, the highest plant was after 16 weeks in all the F0, F2, F3, F5 and F4 treatments. The men plant height up to 4 months, were as follows: F0 (154.33, 150.00, 162.33 and 175.33 cm), F2 (148.67, 155.00, 182.67 and 95.33 cm), F3 (152.67, 173.67, 147.00 and 84.33 cm), F4 (133.00, 148.00, 155.67 and 58.33 cm), F5 (142.67, 155.33, 164.00 and 53.00 cm). The results were significantly different at p<0.01 (HSD tests).

DISCUSSION

Flooding events with partial to complete inundation of plants, can have severe effects on the abundance and distribution of wild plant species in natural ecosystems (Voesenek *et al*., 2004) and on the productivity of crops (Bailey-Serres and Voesenek, 2008). An aqueous environment is stressful to terrestrial plants because of 10^4 fold slower rates of gas diffusion compared with air. The consequent limited exchange of gases such as CO_2 and O_2 dramatically limits photo-synthesis and respiration, respectively. Ultimately, an imbalance between the production and consumption of carbohydrates coupled with an accumulation of toxic metabolic end products proves fatal for most non-adapted terrestrial plants (Bailey-Serres and Voesenek, 2008). Physiological studies have revealed that the gaseous plant hormone ethylene, which rapidly accumulates within flooded organs attributable to the reduced gas exchange underwater, is one of the main drivers regulating both strategies. Indeed, ethylene is considered to be the most reliable and earliest indicator of the flooded status of a plant (Voesenek and Sasidharan, 2013) because, as also shown here, internal oxygen levels can be quite high in submerged photosynthetic tissues, especially when sufficient illumination is present (Mommer *et al*., 2007). Ethylene can either stimulate or suppress growth, depending on the species (Nagai *et al*., 2010) and was also shown to be relevant in submergence survival strategies of rice (*Oryza sativa*) (Xu *et al*., 2006; Hattori *et al*., 2009). It is a long shot to speculate which of the above-listed mechanisms that would have played their roles either individually or in concert on the growth patterns displayed by *S. grossus* when subjected to different depths of inundation and different fertilizer regimes. Notwithstanding, these drivers that may regulate growth strategies of *S. grossus* will serve venues for further research on the scourge.

There is no concrete evidence from the experimental data that *S. grossus* instituted such escape or quiescence strategy to increase population number of ramets, enhanced biomass production or increased plant height with increased water depth. In fact, with increased depths of inundation, population number of ramets decreased, irrespective of fertilizer regimes (Table 1). Such an escape strategy is energetically expensive because it requires considerable amounts of carbohydrates to fuel the rapid growth toward the water surface (Setter and Laureles, 1996). Therefore, escape growth is beneficial only if the flooding event is not too deep to outgrow and if the growth investment is rewarded by restored gas exchange and aerial photosynthesis as the leaves emerge above the water surface (Pierik *et al*., 2009). If the water surface is not reached, survival of escape-driven plants is severely reduced. Deep or transient flood conditions favour species with growth-suppressing behavior upon submergence by limiting carbohydrate consumption and elongation growth, the so-called quiescent strategy (Akman *et al*., 2012). This situation seems prevalent *S. grossus* population subjected to increased depth of flooding or inundation, where mortality rates were increased accordingly.

In this study, *S. grossus* was subjected to varying depths of water levels up to 20 cm and a flood duration of 16 weeks. The plants responded quickly to inundation. However, *S. grossus* plants grown in deeper water levels were unable to grow to the water surface or emerged as such. This observation was similar to the study on *M. aquaticum* but the response might have been different, if the duration of flooding was reduced to a shorter period (Cook and Johnson, 1968), suggesting that this species does not grow well under sustained deep flooded conditions. Light transmittance was 25% in all treatments, which was sufficient to promote submerged plant growth. However, in this study a significant decline in biomass of *S. grossus* and plant length was observed as water levels increased, suggesting that submerged leaves alone cannot sustain growth for long periods of time.

The decrease in growth rates of *S. grossus* at water depth of 20 cm could be due to several reasons. Firstly. it has been reported that optimal photosynthetic rates in *M. aquaticum* occur in its emergent form and therefore it cannot remain as a submerged plant for long periods of time as the photosynthetic rate of the submerged leaves will not be sufficient to support plant growth in the long term (Salvucci and Bowes, 1982). Secondly, it is contended that submerged plant growth is transient and only utilized for short overwintering periods, times of reduced light and temperature (Sytsma and Anderson, 1993), or to survive disturbances in the growing environment. Prolonged exposure to adverse growing conditions will result in reductions in growth and eventually plant mortality. Thirdly, as has been suggested in one study, the presence of algae leads to reduced hydrocarbon content and works as a light insulator and thus prevents the growth of weed (Deng *et al*., 2012). Similar conditions of heavy growth of algae may have helped to reduce the growth of *S. grossus* in our experiments.

There were obvious disparities in the growth of *S. grossus* subjected to different fertilizer and inundation regimes. Devoid of fertilizer application, the sedge displayed time-mediated increase in plant height from 154.33 cm, one month after transplanting to 175.33 cm three months later. These trends were not registered with higher fertilizer applications and increasing depths of inundation.

The two-way interactions between fertilizer and depths of inundation factors were very obvious among plants in the F5 x D4, as measured by plant height. The effects of inundation depths on the plant height of the sedge were very obvious.

What could be the reasons for this poor show of growth by plants of *S. grossus*, despite high rates of fertilizer application? If we could draw parallelism from the studies by Hannual *et al.* (2013) on the response by two *Rumex* spp. when subjected to flooding to help explain the growth disparities displayed by *S. grossus* plants in this study. While flooded environment is lethal for most plant species, flooding-induced metabolic reprogramming specific to *R. acetosa* prevailed, illustrating a survival strategies in anticipation of restriction in gas exchange and mediate an energy and carbon crisis (Hannula *et al*., 2013). *Rumex palustris* uses the early flooding signal ethylene to increase survival by regulating shade avoidance and photomorphogenesis genes to outgrow submergence and by priming submerged plants for future low oxygen stress. It is only possible but yet to be proven, that *S. grossus* may have employed the escape or quiescence strategy as illustrated by both *Rumex* spp. above either flooding-induced metabolic reprogramming with anticipated restriction in gas exchange in order to mediate an energy and carbon crisis, or making use of early signal ethylene for ensuing low oxygen stress.

The escape growth is beneficial only if the flooding event is not too deep to outgrow and if the growth investment is rewarded by restored gas exchange and aerial photosynthesis as the leaves emerge above the water surface (Pierik *et al*., 2009). Deep or transient flood conditions favour species with growth-suppressing behavior upon submergence by limiting carbohydrate consumption and elongation growth, the so-called quiescent strategy (Fukao *et al*., 2006; Akman *et al*., 2012). Indeed, studies show that species with an escape strategy are prevalent on natural sites with frequent shallow and long-term flooding events, whereas those with a quiescent strategy are restricted to sites with deep or short-lasting floods (Voesenek *et al*., 2004).

CONCLUSION

In the case of *S. grossus*, there is a strong evidences from this study that the depth of inundation in excess of 15 cm is deleterious to the growth of the sedge with shorter stature. The results of this study denote that an increased water level significantly decline the biomass and plant length of *S. grossus*. It can be concluded from the study that ramet mortality of *S. grossus* increased with the greater depths of the water level and with increasing NPK concentrations. It would be interesting to pursue further studies on the above lines in order to mechanisms of survival fitness strategy of *S. grossus* when subjected to intermittent or prolonged inundation.

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