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REGENERATION ABILITY OF FRAGMENTS OF *Ludwigia sedioides* (Humb. & Bonpl.) H.Hara: A POTENTIAL INVASIVE PLANT IN SRI LANKA

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ABSTRACT

Ludwigia sedioides (Humb. & Bonpl.) H.Hara is an ornamental aquatic plant recognized as a potential invasive in Sri Lanka. Recently concluded studies revealed that it is naturalized in the wet zone of Sri Lanka. Though this plant has not yet been recognized as an invasive plant elsewhere in the world, the current rate of spread suggests that it might become an invasive plant in the near future in Sri Lanka. Hence, present study was conducted to gain an insight into the ability of L. sedioides to regenerate via fragmentation. Shoots of six different lengths viz. 1, 2, 4, 6, 8 and 10 cm belonging to the two maturity stages (top and stem cutting) were grown in containers arranged in completely randomized design. Survival percentage and plant growth parameters were recorded in each treatment. All the fragments survived and significantly high survival percentages were recorded in all the fragments of top cuttings (70-86%) and fragments of stem cuttings greater than 6 cm. Mean length increments and root dry weights were significantly higher (P<0.05) in top cuttings. Further, mean root numbers were also significantly high in fragments of top cuttings higher than 2 cm. Hence the top cuttings performed better than stem cuttings. However, it is important to note that even a fragment of 1 cm is capable of surviving irrespective of maturity stage. Thus, it is vital to remove all the stem fragments during any attempts in mechanical control. In conclusion, as non-specialized fragments of L. sedioides can be formed throughout the year, it is suggested to exploit other control methods to prevent further spread.

Key words: Aquatic invasive, Fragments, *Ludwigia sedioides*, Propagation.

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INTRODUCTION

Biological incursions are one of the global terrorizations to native biodiversity and ecosystem function (Mac-Neely and Starham, 1997) and this human involved process is associated with migration and persistent establishment incidents related with invading new habitats (Keller and Taylor, 2008). Increasing the number of introductions into novel areas where they are not native have been increased due to the rapid globalization (Mack *et al*., 2000; Khan *et al.,* 2009) and it has become serious economic and environmental challenge worldwide (Pimentel *et al*., 2005). According to many biologists, the threat posed by invasive species to the endangered species and vulnerable natives worldwide is second only to habitat destruction (Wilcove *et al*., 1998).

During past centuries, introduction of non-native species often occurred either accidently or intentionally (Williams *et al*., 2010). Horticulture trade is recognized as an important source of plant introduction, intentionally, via human involvement (Burt *et al*., 2007). The stakeholders of the horticulture trade includes plant explorers, botanical gardens and arboreta, garden club seed exchanges, plant nurseries and the seed trade around the world (Reichard and White, 2001). A small portion of introduced plants, however, escapes from cultivation and become problematic plant in natural areas (Wilcove *et al*., 1998). Scientists estimate that only one in every thousand newly introduced plants in non-isolation regions become invasive in Central Europe (Dollarcker *et al*., 2007). Among escaped horticultural plants, aquatic plants have become serious invaders around the world including Sri Lanka. Many of them have origins in the aquarium trade.

Ornamental aquatic plants in Sri Lanka accounts for over 368 species and cultivars and 76% of these are exotics while 30% of these plants were recorded for their invasive behavior elsewhere in the world (Yakandawala, 2012). Hence there is a greater chance for these plants to become invasive in the future. *Ludwigia sedioides* L. is one such escape initially spotted in a single water body in the wet zone of the country (Yakandawala and Yakandawala, 2007). According to recently concluded studies from the initial water body now it has spread into 36 different pools/ditches and water bodies and recognized as a potential invasive plant in Sri Lanka (Yakandawala *et al*., 2014). However, none of the other countries yet reported this plant as an invasive or potential invasive plant.

Ludwigia sedioides, commonly known as Mosaic flower belongs to the family Onagraceae and native to South America. It is an herbaceous perennial submerged plant with floating leaves. Propagation studies conducted with *L. sedioides* revealed that top cuttings and stem cuttings both have the capability of reproduction and 6 cm fragment is the most suitable cutting type for regeneration (Debarawatta and Yakandawala 2009).

Stem fragments are identified as one of the common vegetative dispersal units in aquatic plants (Riis and Sand-Jensen, 2006). Stem fragments can be produced by disturbances, such as water flow, feeding activity by animals, or by human engagements (Barrat-Segretain, 1996; Madsen and Smith, 1997). Especially mechanical weed control operations can produce a large number of fragments. Regeneration ability of aquatic plant fragments has been documented for several species such as *Egeria densa* (Thiebaut *et al*., 2016), *Lagarosiphon major* (Umetsu *et al*., 2012) and *Mayaca fluviatilis* (Yakandawala and Dissanayake, 2010). According to Riis *et al*. (2009) and Umetsu *et al*. (2012), length of the fragment has a positive relationship with the regeneration ability. Subsequently, management practices of *M. fluviatilis* have also been modified accordingly as studies indicate that 2 cm length stem fragments are capable of giving rise to new plants (Yakandawala and Dissanayake, 2010) and therefore, herbicide control has been effectively tested (Madigan and Vitell, 2012).

As *L. sedioides* is not yet recognized as a problematic plant elsewhere except in Sri Lanka. The objective of the study was to gain an insight into the ability of *L. sedioides* plants to regenerate via fragmentation.

MATERIALS AND METHODS

Location of the Study

The experiment on regeneration ability of fragments of *L. sedioides* was carried out at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura inside a net house (70% shade) during the period from January to August in 2014.

Planting Material and Experimental Design

Planting materials were collected from a naturally occurring population in Pugoda, Gampaha district, Sri Lanka. The shoots were cut into six different fragment lengths (i.e. 1, 2, 4, 6, 8 and 10 cm) to represent two maturity stages of stem fragments (top cuttings and stem cuttings) and these fragments were used as the treatments. Details of the maturity stage, average number of nodes cutting^{-1} and the length of fragments of *L. sedioides* are given in Table-1. Twenty cuttings for each treatment were planted in a container (Diameter x height: 45 × 17 cm) filled with 13 L of water and 2 kg of media (top soil:sand 2:1) and placed inside a net house to provide 80% shade. The containers were laid out in a completely randomized design (CRD) representing four replicates per treatment. The water level of each container was maintained at a constant level throughout the experiment.

Data Recording

The survival of stem fragments of top cuttings and stem cuttings were counted and plant growth parameters *viz*. number of shoots, number of roots, length increment of the fragment, shoot dry weight of the newly formed shoots and root dry weight were recorded six weeks after planting.

Table-1. Details of the maturity stage, average number of nodes per cutting and the length of fragments of *L. sedioides*

Treatment	Maturity stage	Length of Fragment (cm)	Average no. of nodes cutting^{-1}
T 1	Top cuttings	1	41
T 2		2	53
T 3		4	66
T 4		6	71
T 5		8	76
T 6		10	82
T 7	Stem cuttings	1	2
T 8		2	3
T 9		4	8
T 10		6	17
T 11		8	19
T 12		10	24

Data analysis

The root dry weight, shoot dry weight and length increment were subjected to ANOVA and root and shoot number data were subjected to Kruskal – Wallis and Moods Median Tests to compare the effect of the maturity stage of stem cuttings and fragment length.

RESULTS AND DISCUSSION

Survival of Stem Fragments

All the fragments belonging to two maturity stages of different lengths ranging from 1 cm to 10 cm were survived. Significantly high survival percentages were recorded in all the fragments of top cuttings (70-86%) and fragments of stem cuttings greater than 6 cm (Fig. 1).

Significantly lower survival rates were recorded in 1 and 2 cm fragments of stem cuttings followed by 4 cm fragments.

Ornamental aquatic plant industry has shown a steady growth during the past years and Sri Lanka has been a supplier of native and endemic aquatic plants to global demands for decades (Yakandawala *et al*., 2014). Even though the country harbors native aquatics, the industry hunts for popular exotic plants with global demand. Consequently, the industry is involved in propagation of exotics locally for re-exportation (Yakandawala and Dissanayake, 2010). Similar to other countries in Sri Lanka too, some of these plants have escaped the controlled environments and invaded into natural areas and cause irreversible damage to the aquatic ecosystems. One such escape is *L. sedioides* where from a single water body in 2006 (Yakandawala and Yakandawala, 2007), now it has spread rapidly and invaded into 36 different places in the wet zone of Sri Lanka (Yakandawala *et al*., 2014). As up-to-date it has been widely used as an ornamental aquatic and not yet been considered as an invasive in any other country, in depth information on biology, propagation, allopathic effects etc. are not available.

Vegetative propagation is one of the main reproductive mechanisms of aquatic plants of which stem fragments play an important role. Hence the higher rate of fragmentation enhances the rapid dispersal of aquatics between connected aquatic ecosystems (Redekop *et al*., 2016). *Ludwigia sedioides* produces unspecialized stem fragments. Not like specialized fragments such rhizomes, tubers and turions, these fragments can be formed throughout the year.

The results of the present study revealed significant differences in the survival rates between the fragments of two maturity stages; top cuttings and stem cuttings. Irrespective of length, all the fragments of top cuttings showed a significantly high survival rates. However, in the fragments of stem cuttings increase in fragment length increase the survival and only longer fragments (6 cm and above) showed a significantly high survival rates. According to Wu *et al*. (2007), longer fragments possess stronger reproductive capability as longer fragments possess more nodes resting more lateral buds. In *L. sedioides* too longer fragments possess more nodes and compared to stem cuttings, top cuttings possess more nodes (Table 1). According to Heidbüchel *et al*., (2016) and Redekopa *et al*., (2016), the propagule pressure of aquatic plants is enhanced by fragmentation. In *L. sedioides* irrespective of the maturity stage, it is important to note that even a 1 cm fragment is capable of regenerating into a new individual. According to Anderson (1998) and Kuntz *et al*., (2014), though, smaller fragments are recorded to have a low viability; a high regeneration capacity was recorded in three *Elodea spp.* Only small stem fragments are required by aquatic macrophytes to regenerate into new populations as most of them possess meristems distributed closely along their stems (Riis and Sand-Jensen, 2006).

Growth performance of Stem Fragments

Length Increment

The lengths of the fragments were not increased in stem cuttings. However, length increment was observed in fragments of top cuttings. Fragments of 4 and 6 cm length recorded the significantly high increments followed by 8 and 10 cm fragments. The smallest fragment (1 cm) recorded the lowest increment (Table-2).

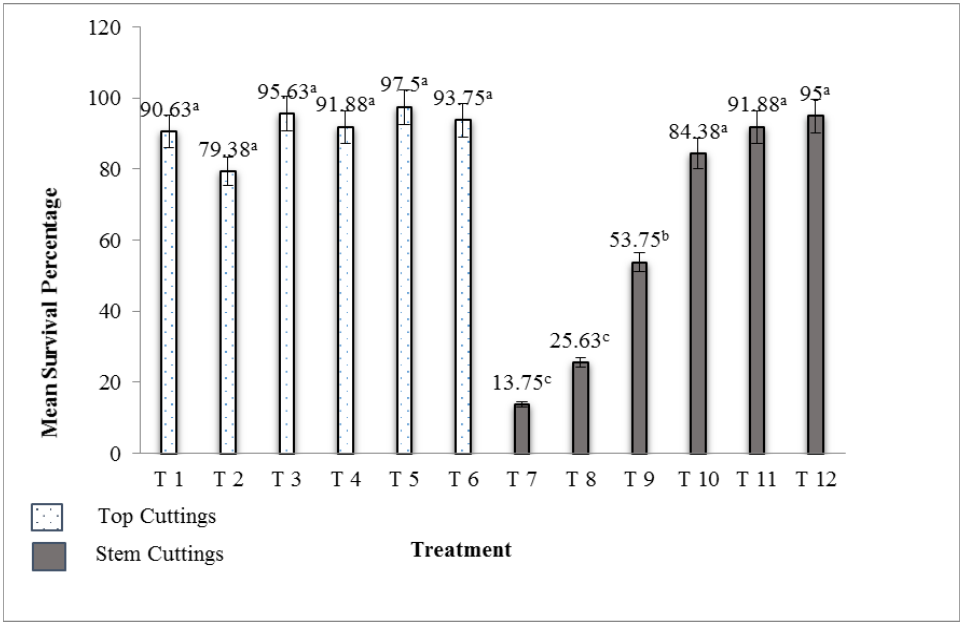

Figure 1. Survival percentage of fragments (±SE) under different treatments.

Note: T1 & T7 = 1 cm; T2 & T8 = 2 cm; T3 & T9 = 4 cm; T4 & T10 = 6 cm; T5 & T11 = 8 cm and T6 & T12 = 10 cm, top & stem cuttings, respectively.

High length increment recorded in the fragments of top cuttings is due to the presence of a terminal bud. In nature, *L. sedioides* is capable of producing long stalks and it produce floating leaves hence it has a potential to survive in deep waters. While high root numbers and dry weight of top cuttings is due to the formation of more roots. It will ensure the subsequent survival of the fragments. According to Barrat-Segretain *et al*. (1999), roots produced from stem fragments once separated from the mother plant facilitate the subsequent establishment in a favourable environment.

Root Dry Weight

Root development was observed in all the fragments. Significantly high root dry weights were recorded in all the fragments of top cuttings compared to stem cuttings and this is due to the presence of higher number of nodes in top cuttings. Top cuttings with 6 cm length recorded the highest root dry weight which is significantly different from that of all the other treatments (Table-2).

Shoot Dry Weight

All the fragments of stem cuttings produce new shoots while only 1, 4 and 6 cm long fragments of top cuttings produce new shoots from the stem parts below the rosette. Shoot dry weights of fragments were significantly high in stem cuttings compared to top cuttings except in 1 cm long fragment. The significantly high shoot dry weight was recorded in 6 cm long fragments of stem cutting (Table-2).

Shoot Number

Only longer fragments of stem cuttings *viz*., 6, 8, and 10 cm produced shoots (Table 3). Whereas new shoot formation was not observed in fragments of top cuttings and in smaller fragments of stem cuttings.

Table-2. Growth performance of different fragment lengths in different maturity stages of *L. sedioides*

Maturity stage	Length of Fragment (cm)	Mean length increment (cm)	Mean shoot dry weight (g)	Mean root dry weight (g)
Top cuttings	1	14.682e	0.00296d	0.20d
	2	17.918d	0.00000e	0.19d
	4	23.641a	0.00010e	0.53c
	6	22.946a	0.00161e	9.86a
	8	22.163b	0.00000e	0.51c
	10	21.803c	0.00000e	0.79b
Stem cuttings	1	0.000 f	0.00200d	0.02f
	2	0.000 f	0.02324c	0.02f
	4	0.000 f	0.04386c	0.08e
	6	0.000 f	0.32247a	0.05f
	8	0.000 f	0.03313c	0.07e
	10	0.000 f	0.08985b	0.03f
P value		<0.001	<0.001	<0.001

Root Number

The median root number increased with the increased fragment length in both maturity stages except in 10 cm long top cuttings. While significantly higher root numbers were recorded in 8 cm long top cuttings compared to other treatments (Table-3). Root development was not observed in 1 and 2 cm fragments of stem cuttings.

Table-3. Shoot and root number of different fragment lengths in different maturity stages of *L. sedioides*

Maturity stage	Length of fragment (cm)	Median of shoot number	Median of root number
Top cuttings	1	0.00^{b}	8.50^{d}
	2	0.00^{b}	11.0^{c}
	4	0.00^{b}	13.0^{b}
	6	0.00^{b}	14.0^{b}
	8	0.00^{b}	16.0^{a}
	10	0.00^{b}	12.0^{c}
Stem cuttings	1	0.00^{b}	0.00^{g}
	2	0.00^{b}	0.00^{g}
	4	1.00^{a}	2.50^{f}
	6	1.00^{a}	6.00^{e}
	8	1.00^{a}	8.00^{d}
	10	1.00^{a}	11.0^{c}
P Value		<0.001	<0.001

Mean shoot number and shoot dry weight was significantly higher in fragments of stem cuttings compared to top cuttings. Wu *et al*. (2007), also noted that compared to fragments of top cuttings, the stem fragments had the higher ability of producing lateral shoots. The formation of new shoots is vital for stem cuttings for their survival as it does not possess any leaves to photosynthesis. Hence, these fragments might have used the stored food reserves in the stem until they form shoots. Hence, low survival rates of small stem fragments could be due to the above fact.

Ludwegia sedioides is a submerged plant which produces floating leaves and attached to the water body by roots. Understanding the methods of regeneration is an important step towards the maintenance of exotics and its dispersal (Richardson *et al*., 2000). This knowledge is vital in the management of invasive species hence cannot be achieved without adequate information on reproduction and subsequent spread of the invader (Forman and Kesseli, 2003). The current study discloses the propagation capability of two maturity stages (viz. top and stem cuttings) of stem fragments in *L. sedioides*. Among them, top cuttings recorded highest survival rates and significantly high length increments and high root growth parameters. Hence terminal fragments have a comparatively higher advantage for survival and subsequent regeneration. Further, according to Wu *et al*. (2007), top cuttings exhibit longer dispersal distances and this leads to establishment of the plant in new destinations.

Propagule pressure is a main factor responsible for the establishment success of invading plants, especially during the initial establishment phase (Liu *et al*., 2014). Several studies have indicated a positive relationship between propagule pressure and success of the invasion (Colautti *et al*., 2006). The present study revealed a high propagule pressure in *L. sedioides* under laboratory conditions. Further studies are necessary to validate whether or not similar patterns occur in field populations of *L. sedioides*. Nevertheless, the findings of the present study contribute to the explanation of the potential invasiveness of *L. sedioides.*

CONCLUSION

A high propagule pressure was witnessed in *L. sedioides* and it is important to note that even a 1 cm fragment has the ability to regenerate into a new plant. The significantly high regeneration ability was recorded in fragments of top cuttings compared to stem cuttings. Hence, as all the fragments of different lengths irrespective of maturity stage were capable of surviving, it is important to remove all the fragments during any attempts in mechanical control. As fragments can be formed throughout the year immediate action is suggested to prevent further spread.

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