Clonal integration of the invasive plant *Wedelia trilobata* (L.) Hitch in stress of flooding type combination

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Abstract

The clonal invasion of *Wedelia trilobata* (L.) Hitch has spread to riverside and edges of mangrove areas, which leads to the formation of flooding-stressed areas such as waterlogged and submergence. This study purpose to investigate the clonal integration mechanism of *W. trilobata* in stress of flooding type combination. This study was conducted in greenhouse with four combinations of flooding treatment on mother ramet (MR) and daughter ramet (DR) for 25 days. Several parameters were measured are shoot growth, relative growth rate (RGR), biomass, biomass allocation, adventitious root growth, and lenticel hypertrophy. The highest clonal performance was observed for the combination of field capacity (MR)-waterlogged (DR). The lowest performance was observed for the combination of waterlogged (MR)-submergence (DR). There were decreases in the shoot growth, RGR, and biomass allocation in mother ramet. However, adventitious root growth and lenticel hypertrophy increased in daughter ramet. The increase of flooding pressure suppresses the performance of clonal plants. Clonal integration buffered clonal plants by improving the performance of daughter ramet in the combination of flooding type. The clonal integration has facilitated *W. trilobata* invasion in inundated areas.

Introduction

Invasive plants are often aggressive weeds that spread rapidly and disrupt the community structure of native plants by replacing them. The invasion of these plants affects ecosystem composition and diversity, such as lead to the alteration of micronutrient, hydrology, increase the frequency of land fires.[1] Furthermore, the ecosystem disruption caused by foreign species invasion can result in economic sector collapses, environmental damage, and health problems.[2]

The majority of invasive plants are clonal plants consisting of ramets connected by stolons or rhizomes.[3] Ramets include mother and daughter ramets or old and young ramets. The main character of clonal plants is clonal integration, partitioning resources between ramets.[4] Stressed environmental conditions could affect to resource translocation from unstressed ramet to be stressed ramet. Therefore, increasing number of resources will enhance the recipient ramets survival.[5] The influence of clonal integration on ramets performance can be measured from growth, biomass allocation and morphological characters.[6] The performance improvement facilitates the invasive plants in the environmentally stressed area.[7]

Flooding stress on plants is categorized based on the location of water accumulation. When the flooding occurs in the rooting system it is termed waterlogged conditions. However, if the flooding is found all over the plants' body*, i.e*, the plants are sunk in the water, it is called submergence.[8] This condition brings a negative effect on the plant, *i.e*, inhibits gas diffusion such as O_2 and CO_2, alters soil electrochemical components, energy crisis, ROS (Reactive Oxygen Species) production, and water deficiency.[9]

Wedelia trilobata (L.) is a clonal plant included in 100 worst invasive species worldwide.[10] It is reported that *W. trilobata* threatens vegetation biodiversity in several areas including ecotone between terrestrial and mangrove ecosystems.[11,12] *W. trilobata* has invaded riparian (riverside) and mangrove areas.[13] In addition, both waterlogged and submergence flooding types have been commonly found in riparian zones.[14]

The clonal plants spread horizontally through heterogeneous environmental conditions such as nutrient availability.[3] Therefore, clonal plants can experience in stress of flooding type combination on their constituent ramets. Ramets can be found in waterlogged conditions and others under submergence condition, even if the same clonal plant.[12] Stated that the clonal integration strategy plays a pivotal role in *W. trilobata* invasion. Accordingly, this study has been conducted to investigate the clonal integration of W. trilobata under flooding type combinations.[12]

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Materials and Methods

Sample preparation

W. trilobata samples were taken from Semarang, Central Java, Indonesia. The clonal plants of *W. trilobata* were propagated vegetatively for two years. The age of the clonal plants was 3 months after plantation. Stolons (2 nodes) were planted in sand and compost mixture (2:1). After 1 month, stolons were transferred into plastic pots with river sand medium. Seedlings were fertilized every week with 32:10:10 N:P:K liquid fertilizer and watered every day until seedling stolon length was 50-55 cm. Daughter ramets were produced by planting the node (the second node from apical stolon) into a separate plastic pot containing the river sand medium. Roots grew 10 days after planting.

Flooding stress treatment

Mother ramets were taken when the stolon length was about 51.79 ± 1.99 cm with the number of leaves 14 and leaf area 142.36±3.99. For daughter ramets were used stolon length was about 36.68 ± 2.08 cm with a number of leaves around 6-8 and leaf area 67.55 ± 2.47. Twenty clonals were divided into four combinations of flooding stress (5 clonals for each treatment group). Specifically, there were six clonals in control group, which were measured at the beginning of treatment (H-0).

Measurement

Destructive measurements were done to mother and daughter ramets parameters before flooding stress treatment (H-0) to obtain primary data of leaf area, biomass, and biomass allocation. The parameters were measured are the root $biomass_{(mother/daughter)}$, shoot $biomass_{(mother/daughter)}$, clonal biomass, shoot mass ratio (SMR), and root mass ratio (RMR).

Stolon length and the number of leaves were measured through non-destructive measurements. At the end of treatment both mother and daughter ramet parameters were measured, *i.e.,* healthy and damaged leaves number, leaf area, stolon length, stolon increase rate, ramets biomass (root $biomass_{(parental/seedlings)}$ and shoot $biomass_{(mother/daughter)}$), biomass allocation ($SMR_{(mother/daughter)}$, $RMR_{(mother/daughter)}$, and $ArMR_{(mother/daughter)}$ = adventitous root biomass/total of clonal biomass), and relative growth rate (RGR), including root RGR, crown RGR, and clonal RGR.

In addition, morphological characters were also observed to obtain information about clonal adaptation under flooding stress such as the number of adventitious roots (ARs) along with its length and wet mass as well as lenticel hypertrophy.

Statistical analysis

One-way analysis of variance (ANOVA) and LSD test with significance P<0.05 were conducted to analyze clonal integration and flooding stress type influence toward shoot growth, RGR, biomass, biomass allocation, and seeds morphological characters. The analyses were performed using SPSS 16.0 (SPSS, Chicago, Illinois, USA).

Results

Shoot growth

The flooding types combination treatments for 25 days affected the shoot growth of clonal plants. There are significant differences in the shoot growth parameters except for stolon length of mother ramets (Table 1). Treatment FC-W group caused highest shoot growth supported by the highest number of healthy leaves and leaf area. However, treatment FC-S caused the highest damaged leaves, around 7.60 ± 0.24 per plant, leading to the lowest number of healthy leaves and leaf area (Table 1).

Moreover, daughter ramets growth was significantly influenced by the flooding type combination (Table 1). All daughter ramets growth parameters in each flooding type combination were significantly different (P<0.05). Treatment FC-W group showed the highest increase of stolon length, healthy leaves, and leaf area, as it had the lowest number of damaged leaves among the other treatments. On the other hand, W-S treatment had the lowest daughter ramet growth with the highest damaged leaves number, around 6.00 ± 0.00.

Relative growth rate

There were significant differences in RGR parameters among all flooding type combination (Figure 1). Overall, the FC-W group had the highest RGR, while W-S had the lowest. In addition, FC-S group showed the lowest shoot RGR of mother ramets and root RGR of daughter ramets.

Biomass

The flooding type combination increased the biomass of clonal, mother and daughter ramets, which were significantly different among all treatment (P<0.05) (Figure 2). The highest biomass was obtained from FC-W group (biomass of clonal, mother and daughter ramet). The W-S group had the lowest seedlings biomass and total clonal number (Figure 2).

Biomass allocation

The biomass allocation of each treatment was significantly different (P<0.05) including RMR, SMR, ArMR of both mother and daughter ramets (Figure 3). The pattern biomass allocation was similar among the FC-W, FC-S, and W-W treatments. The highest biomass allocation was observed in daughter ramet shoots followed by mother ramet shoots, mother ramet roots, daughter ramet roots and adventitious roots of daughter ramet. The lowest biomass was allocated to mother ramet adventitious roots. In addition, the W-S treatment group showed the highest biomass allocation in mother ramet shoots, but the lowest allocation was found in mother ramet adventitious roots.

Morphological characters

Overall, morphological adaptations were observed in all treatment groups, but

Table 1. The influence of flooding type combination on stolon length, an increase of stolon length, healthy leaves number, number of damaged leaves and leaf area.

Ramet	Parameters	FC-W	FC-S	W-W	W-S
Mother	Stolon length (cm)	51.50±0.77a	52.34±0.91a	52.18±0.63a	53.70±1.13a
	Increase of stolon length (%)	1.25±0.21a	1.24±0.15a	1.05±0.18a	1.37±0.19a
	Healthy leaves number	13.40±0.24a	6.80±0.37d	12.40±0.24b	9.60±0.24c
	Damaged leaves number	0.60±0.24d	7.60±0.24a	1.60±0.24c	4.40±0.24b
	Leaf area (cm^2)	137.74±2.60a	47.85±2.39d	125.18±2.20b	103.04±2.61c
Daughter	Stolon length (cm)	130.78±1.27a	67.76±1.95c	108.40±1.48b	47.80±0.93d
	Increase of stolon length (%)	256.29±6.74a	89.55±3.47c	188.19±5.54b	31.03±0.8d
	Healthy leaves number	15.60±0.24a	10.60±0.40c	14.40±0.24b	7.20±0.50d
	Damaged leaves number	2.40±0.24c	5.40±0.40a	3.60±0.24b	6.00±0.00a
	Leaf area (cm^2)	155.45±1.85a	92.18±1.78c	136.60±2.42b	63.84±3.98d

Mean±SE (n=5) in different letters show significant differences (P<0.05) between flooding stress types.

the adaptations were mostly found in mother ramet at the W-W treatment group involving adventitious roots and lenticel hypertrophy. Changes were least observed in FC-W treatment group. There was a significant increase in the development of daughter ramet adventitious roots in FC-S and W-S, but a decrease in the W-W and FC-W treatment groups. Furthermore, the highest daughter ramet lenticel hypertrophy occurred in W-W group by around 34.60 ± 1.36. Meanwhile, the lowest lenticel hypertrophy occurred in FC-W group or 2.40 ± 0.24 (data not shown).

Discussion

Wedelia trilobata (L.) is perennial herb originating from South America that is now widely spread into South China, Fiji, Sri Lanka, Micronesia, and Indonesia.[13] This plant has been known as an invasive weed that is supported by its clonal integration as the main characteristic of the clonal plant. The clonal integration between ramets facilitates resources distribution under environmental stress and influences the clonal performance.[15]

The highest clonal biomass was found in FC-W flooding type combination, followed by W-W, FC-S, and W-S (Figure 2). Likewise, clonal relative growth rate results showed the same pattern as biomass (Figure 1). The waterlogged condition leads to anoxia and hypoxia conditions that further cause an energy crisis in the plant. This condition inhibits root development and also prevents water and nutrients to be absorbed.[16]

Under submergence conditions light radiation is blocked, CO2 diffusion and photosynthesis decreases in the shoot, further causing anaerobic conditions in the root area.[8] The strongest flooding pressure was administered to the W-S group, while the weakest given to FC-W group (Table 2). It can be seen in Figure 1 and Figure 2, the increase of flooding stress on ramets reduced the clonal performance. Xiao *et al.* reported that water depth in flooding stress treatment to daughter ramet of *Spartina alterniflora* leads to the decrease of shoot biomass and clonal rhizome.[17] Alkaline stress treatment to *Leymus chinensis* daughter ramet leads to biomass decrease on ramets and clonal level.[18]

Flooding stress is an environmental stress that restrains live resource availability such as nutrients, gas diffusion, and light for photosynthesis.[19] The flooding type combination treatment caused differences in resource availability between parental seeds and seedlings, which led to a variety of biomass allocation in each treatment (Figure 3). The most significant differences were observed in the W-S treatment group, where the highest biomass

Table 2. Experimental design.

Combination of flooding type	Flooding types	
	Mother ramets	Daughter ramets
FC-W	Field capacity	Waterlogged
FC-S	Field capacity	Submergence
W-W	Waterlogged	Waterlogged
W-S	Waterlogged	Submergence

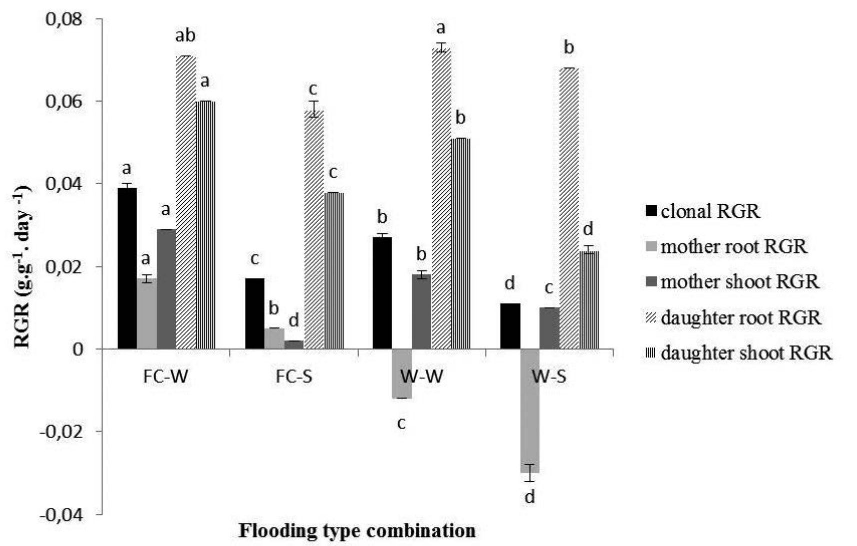

Figure 1. The influence of flooding type combination on clonal RGR, root RGR and shoot RGR (mean±SE, n=5) (P<0.05).

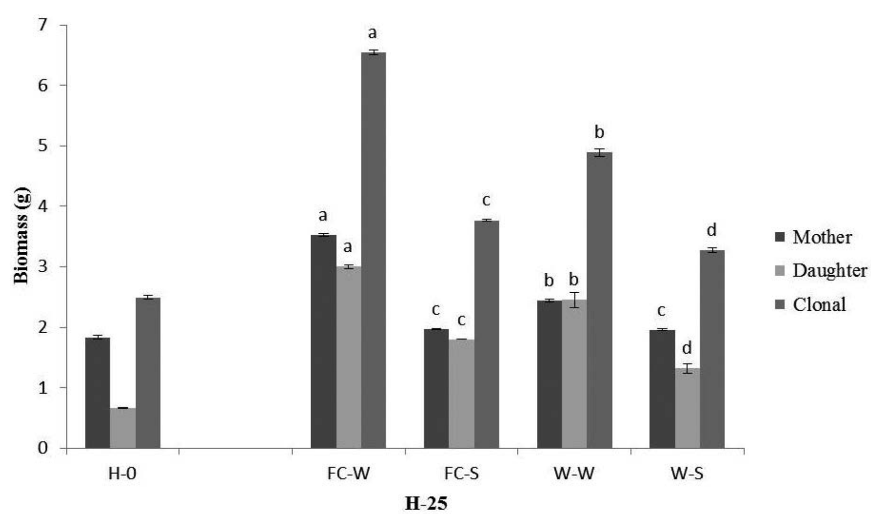

Figure 2. The influence of flooding type combination on biomass. H-0 (mean±SE, n=6) is the primary biomass before treatment (P<0.05).

allocation was found in the shoots of mother ramet followed by the allocation to the shoots of daughter ramet. The results also showed a decrease in the allocation of biomass in the shoots and roots of the mother ramet, while there was an increase of biomass allocation to roots, shoots and adventitious roots of daughter ramet at FC-W, FC-S and W-W treatment.

The relative growth rate of root and shoots in mother ramet were lower than in daughter ramet. Moreover, there were decreases in shoot development of all mother ramets. This was related to daughter ramets growth that led to the increase of biomass allocation in the shoot, root, and adventitious root. Again, similar results were found in other clonal plants. As has been reported by Xiao *et al.*, there were increases found in shoot length and biomass accumulation of *S. alterniflora* seedlings under flooding stress.[7] Several studies show that *Fragaria orientalis* clonals were survived from drought stress by the support of ramet donors with more resources availability.[20] Resources translocation was observed in *Potamogeton perfoliatus* daughter ramets under shade stress, in which the older daughter ramets give their resources to the younger daughter ramets as they need more resources to grow.[21] Clonal integration facilitates the translocation of resources from rich-resourced ramets to poor-resourced ramets or from source to sink. Moreover, the results show that the direction of resource transport begins in old ramets to young ramets or acropetal. It can be seen in W-W group that had homogenous environmental stress between seedlings. The ramets survival under flooding stress shows that clonal integration strategy supported ramets performance in the heavier stress condition.[5]

For non-clonal plants, waterlogged and submergence conditions inhibit root growth and biomass allocation into the root.[18,22] These plants tolerate the stress by the formation of adventitious root and lenticel hypertrophy.[23,8] The adventitious roots are formed by aerenchyma cells to support root function as the lenticel hypertrophy increases O_2 diffusion into root cells.[23]

In the waterlogged flooding stress, the root growth rate of mother ramet decreases, but it increases in daughter ramets. The root growth rate of daughter ramets (FC-S and W-S) under submergence flooding stress decreased while the adventitious root increased compared to waterlogged treatments (FC-W and W-W). Moreover, the highest lenticel hypertrophy was found under W-W treatment, followed by W-S, FC-S, and FC-W (Table 2). The growth of adventitious roots in submergence treatments is a strategy to overcome the decrease in root performance. Likewise, the high rate of root growth in the daughter ramets (W-W) due to the development of lenticel hypertrophy. Overall, the clonal integration of *W. trilobata* under flooding type combination leads to the occurrence of morphological adaptation on daughter ramets by forming adventitious root and lenticel hypertrophy. This supports the classical theory that biomass will be allocated mostly to organ with least resources.[24]

The highest shoot growth and biomass of daughter ramets were observed in FC-W group, which is followed by W-W, FC-S and W-S (Table 1; Figure 2). It indicates that submergence flooding stress decreased the performance of daughter ramets. One of the strategies of plant adaptation towards submergence flooding stress is "the low oxygen escape strategy" (LOES) or increasing respiration and photosynthesis by carbohydrate utilization and increasing cellular metabolism to obtain light and gas (O_2 and CO_2).[8] Aerobic conditions under waterlogged flooding stress allow the shoot to perform photosynthesis as the photosynthate will be used in shoot growth (increase stolon length, number, and area of leaves) (Table 1). Therefore, some plants have been reported using LOES strategy, such as *Rumex palustris* elongates its petiole,[25] *Rorippa amphibia* elongates its stem and *Alternanthera philoxeroides* elongates its internodes,[26] *S. alterniflora* clonal elongates its shoot of daughter ramet to overcome submergence.[7]

The increase of stolon length of FC-S and W-S group were 89.55±3.47% and 31.03±0.8%, respectively, lower than the FC-W and W-W groups (Table 1). However, some of the increase of stolon length can appear above the water surface. Regarding this, the clonal integration improves daughter ramets tolerance under submergence flooding stress through LOES strategy. Moreover, the horizontal growth of *W. trilobata* is influenced by daughter ramets growth since the relative growth rate in all treatment groups is higher in daughter ramets than in mother ramets.

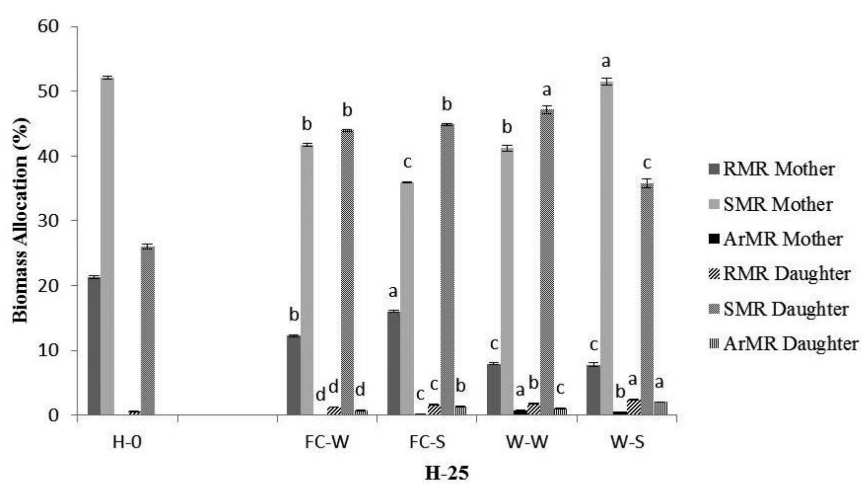

Figure 3. The influence of flooding type combination on biomass allocation. H-0 (mean±SE, n=6) is the primary biomass before treatment (P<0.05).

Conclusions

Clonal integration supports plant survival under stress of flooding type combination by improving daughter ramets performance. The improvement of daughter ramets performance includes root and shoot growth, biomass allocation, morphological adaptation (adventitious root and lenticel hypertrophy) and LOES strategy. Clonal integration strategy has facilitated *W. trilobata* invasion in areas prone to flooding.

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