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NOTE AND COMMENT

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# Time is on our side: the importance of considering a recovery period when assessing flooding tolerance in plants

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**Abstract** There is wide consensus about the significance of monitoring plant responses during flooding when evaluating specific tolerance. Nonetheless, plant recovery once water recedes has often been overlooked. This note highlights the importance of registering plant performance during a recovery phase. Two opposite types of plant growth responses, during and after flooding, are discussed. It is shown that an apparently poor performance during flooding does not necessarily involve a reduced tolerance, as plants can prioritize saving energy and carbohydrates for later resumption of vigorous growth during recovery. Conversely, maintenance of positive plant growth during flooding can imply extensive depletion of reserves, consequently constraining future plant growth. Therefore, to accurately estimate real tolerance to this stress, plant performance should be appraised during both flooding and recovery periods.

**Keywords** Flooding stress · Recovery period · Root recovery · Use of reserve carbohydrates

## Introduction

Plant responses to flooding, ranging from soil waterlogging to complete submergence, have been studied and reviewed extensively. There are recent reviews on anatomical aspects regarding root aerenchyma generation (Seago et al. 2005; Striker et al. 2007), plant internal aeration (Colmer 2003; Wegner 2010), plants' ability to photosynthesize under water (Mommer and Visser 2005; Colmer et al. 2011), molecular aspects of oxygen sensing (Licausi et al. 2011; Sasidharan and Mustroph 2011; Bailey-Serres et al. 2012), intra-specific morphological variation in leaf/petiole elongation (Chen et al. 2011; Huber et al. 2012) and, finally, identification of strategies developed by plants in response to submergence caused by flooding (i.e.. escape vs quiescence; Bailey-Serres and Voesenek 2008, 2010; Manzur et al. 2009; Hattori et al. 2010; Striker 2012). So, it can be stated that much knowledge has been gained in recent years leading to an understanding of what happens to plants during flooding periods, and how they respond to such stress. In addition, some methodological aspects of flooding experiments—like typical flooding duration, age of plants when they are subjected to flooding, presence/absence of competition among plants during flooding—have been examined recently (Striker 2008). Despite all the above, plant responses after water subsides, defining their recovery capacity, have often been overlooked (but see Malik et al. 2002; Striker et al. 2011, 2012). Therefore, conclusions on plants tolerance to flooding have been, in too many cases, based on (and circumscribed to) their responses during or immediately after the stress period. In addition, in cases when recovery period was assessed, in particular in experiments involving complete submergence (see Luo et al. 2009, 2011), the ascribed flooding tolerance is the sum of plant behaviour both during and after the stress. As a result, the distinct effects of the two phases cannot be distinguished. Thus, the focus of this note is on highlighting the importance of considering a recovery period after water excess treatments—in which plant responses also need to be monitored—when assessing real flooding tolerance in plants. In addition, the significance of quantifying root recovery during the recovery period is also discussed, rather than considering only shoot mass accumulation.

## Tolerance to flooding: conclusions depend on considering plant recovery

It is important to note that the case presented is theoretical and, in order to be valid, some assumptions have to be taken into account. First, a constant relative

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growth rate (RGR) is assumed during the whole experimental period for biomass accumulation of plants (e.g., RGR of 3 % on a daily basis as the equation slope for the control plant in Fig. 1). This is a reasonable simplification for a relative short period of growth during the vegetative growth young plants ('classical approach' for plant growth analysis, see Poorter 1989). Second, plant biomass is used as the main variable in determining flooding stress-tolerance, as it integrates all ecophysiological processes leading to growth (Nakai et al. 2009; Li et al. 2011), while 'stress' is defined as any environmental factor that restricts growth (sensu Grime 1989). Third, it is assumed that the use of reserve carbohydrates is essential in defining growth during (and importantly after) flooding. There are several works supporting this last assumption for a great variety of plant species, including both wild and crop species (see review by Bailey-Serres and Voesenek 2010, and references therein).

Two different scenarios are presented regarding plant behavior during the flooding period (Fig. 1). In the first, the flooded plant continues growing during flooding, but at a lower rate than in a well drained soil (e.g., 40 % of controls in the A-type response in Fig. 1b). In this case, plant growth is sustained, for the most part, by using carbohydrate reserves (Fig. 1c), and, to a lesser extent, through current carbon assimilation. These are common responses, reported for several plant species, where the plant's efforts are concentrated on growing during the period of anaerobic conditions caused by flooding, at the expense of reserves consumption (starch and soluble sugars). Examples of this behavior have been found for *Oryza sativa* cultivars (Setter and Laureles 1996), adult plants of the legume *Lotus tenuis* (Striker et al. 2011) and

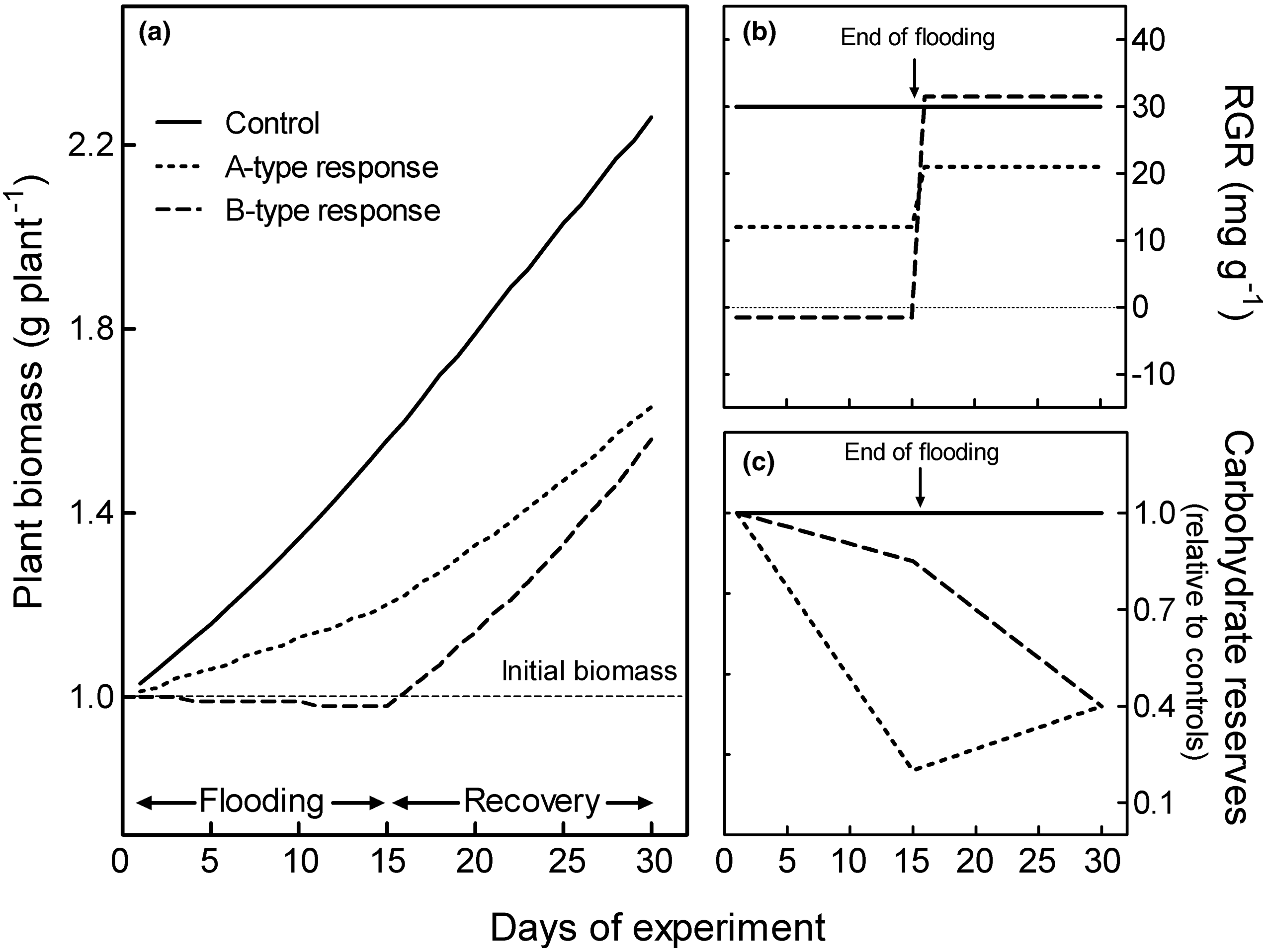

**Fig. 1** Hypothetical scenarios of **a** plant biomass accumulation, **b** relative growth rate (RGR) and **c** carbohydrate reserves (relative to controls) of two different types of plant responses during and after flooding. Flooding and recovery periods both last 15 days. Flooding duration is representative of experiments performed in seedlings (Striker 2008). *Vertical arrows* (**b**, **c**) indicate the end of flooding. Parameters used for biomass accumulation calculations were: initial plant biomass: 1 g plant$^{-1}$; control plant has a constant RGR, equivalent to 3 % of its biomass on a daily basis (**b**); A-type response plant has a growth rate of 40 % of controls during the 15 days of flooding, consuming 80 % of carbohydrates, and it has an RGR that is 70 % of controls during the 15 days of recovery, restoring its carbohydrates levels to 40 % (**b**); B-type response plant has a negative growth rate of 5 % (net biomass loss) with respect to controls in the first 15 days with minimal consumption of carbohydrates (15 %), and a 5 % higher RGR than that of controls during the last 15 days due to intense use of stored carbohydrates for re-growing (final level of 40 %). Data on RGR and carbohydrate reserves consumption are in line with literature values (e.g., supporting information in Striker 2008 and Poorter et al. 2012)

the weed *Rumex palustris* (Voesenek et al. 2006; Chen et al. 2009, 2011), among others. This plant behavior seems to be particularly important under shallow flooding, where plant growth maintenance facilitates leaf elongation and the rapid emergence of leaves above water (Striker et al. 2008). In this respect, it should be noted that flooded plants can elongate their aerial organs more than controls plants, with the result that their longer leaves/petioles can emerge from the water, but they accumulate biomass at a lower rate than controls (i.e., longer but lighter leaves, see Kawano et al. 2009 for rice; Chen et al. 2009 for *Rumex palustris*). A second example of plant behavior also reported in the literature is that flooded plants can suspend their growth temporarily during the flooding period (B-type response in Fig. 1b) by slowing down their metabolism, saving energy and maintaining high carbohydrate reserves (Fig. 1c). Moreover, in such cases plant biomass can drop slightly (Fig. 1a) as a result of the death of fine roots and submerged leaves, both known responses triggered by ethylene accumulation in tissues (Jackson 2008). Examples of this behavior have been found in *Oryza sativa* (e.g., cv. FR-13A, Setter and Laureles 1996; Bailey-Serres and Voesenek 2010), *Ranunculus repens* (Lynn and Waldren 2003), *Rumex crispus* (Voesenek and Blom 1989) and seedlings of *Lotus tenuis* (Striker et al. 2012) among others. This plant behavior is more likely to be associated with deeper flooding, where leaf growth/elongation does not ensure a rapid emergence from water of pre-submerged leaves. Now, the logical question arising is: what can be concluded if the experiment ends immediately after flooding? Certainly, plants with A-type response performance will be regarded as flood-tolerant, whereas plants exhibiting the B-type response will be considered as flood-sensitive (or poorly tolerant). Being conservative, it must be stated that more than one-half of papers on flooding—including some of my own authorship—did not include a recovery phase (see Striker 2008), so that conclusions definitely end at this point.

The conclusions drawn may change if plants are allowed to grow into a recovery phase after they have experienced a similar flooding regime (Fig. 1). It is assumed that, after withdrawal of flood waters, the level of reserve carbohydrates remaining can influence plant growth during recovery (Striker et al. 2011). The availability of remaining reserves is dependent on how plants have used carbohydrates to deal with the energy crisis during flooding, where aerobic metabolism for energy production shifts to the much less efficient anaerobic/fermentative pathways (Bailey-Serres and Voesenek 2008). Hence, a beneficial plant performance after flooding depends greatly on having a high content of reserve carbohydrates allowing the plant to resume a vigorous growth. In the proposed example (Fig. 1a–c), A-type response plants had depleted carbohydrate reserves during flooding (Fig. 1c), and thereafter, their growth rate remained lower than that of controls (but slightly higher than that developed in the stressing flooding period; Fig. 1b). This low growth rate after flooding can be associated, along with lower carbohydrate levels for re-growing, with other plant responses. Some of these responses can be due to sudden re-exposure to ambient oxygen levels, resulting in increased formation of reactive oxygen species (ROS) and other metabolites harmful to plant tissues (Kawano et al. 2002; Blokhina et al. 2003). Also, after flooding, plants are exposed to high light irradiances, which can damage the photosynthetic apparatus (i.e., photoinhibition; Osmond 1994) of previously submerged leaves acclimated to low light conditions. This photoinhibition can hamper post-submergence recovery of photosynthesis. In addition, tissue dehydration is (paradoxically) another factor contributing to post-submergence damage (Voesenek and Blom 1989; Setter et al. 2010). Regardless of having experienced excessive water content in the soil, after flooding, some plants exhibit symptoms of water deficit such as wilted leaves. These symptoms could be due to a reduced hydraulic conductance of previously flooded roots (Colmer and Voesenek 2009). Hence, a diminished water uptake by roots and associated increase in stomatal closure to minimize transpiration can also cause a reduction in carbon fixation after flooding. So, having survived flooding, plants returning to aerial conditions are faced with a new set of stress factors during recovery. For these reasons, it is crucial to monitor plant responses during the recovery phase.

By contrast, B-type response plants maintained a high level of carbohydrates (Fig. 1c), which allowed them to resume plant growth at high rates, even slightly higher than under control conditions (Fig. 1b; see also a similar response for *Lotus tenuis* seedlings in Striker et al. 2012). Then, after the recovery period: what can be concluded about the tolerance of plants showing each type of response? Here, both A or B types of plant response (Fig. 1) lead to similar biomass accumulation when evaluated at the end of the experiment. Therefore, in the proposed scheme, plant tolerance to flooding does not seem to differ much when a recovery period is considered, regardless of the way in which the plant has dealt with this stress (comparison between 'A' and 'B' response types). This exercise highlights, in a simple manner, the importance of including a recovery phase for plants after the stress period imposed by flooding in order to reach accurate conclusions about plant tolerance to this stress. Applying a pragmatic approach, a recovery period of similar length to experimental flooding is suggested as a rule of thumb. However, when thinking more carefully about the set-up of a flooding experiment, biologically relevant parameters such as the growth rate of plants under non-limiting conditions, or the ontogenetic age of plant material (among others), should be considered for determining the most appropriate length of recovery phase according to our purposes.

## Root recovery: the hidden half of plant recovery after flooding

Roots are affected directly by soil anaerobic conditions due to flooding. For this reason, in general terms, during an event of flooding, root growth is more affected by soil water excess than shoot growth (Colmer and Voesenek 2009). In addition, plants that grow during a flooding period (A-type plant response in Fig. 1) prioritize shoot growth and leaf/petiole elongation (Chen et al. 2009, 2011; Huber et al. 2012). In this way, plants maximize the leaf area exposed above water, ensuring the capture of oxygen by leaves (Striker et al. 2008) and facilitating root aeration (Colmer 2003). As a result of such preferential allocation of carbon to shoots—and the discontinuance of root growth—there is an increase in the shoot-to-root biomass ratio in flooded plants (Nakai et al. 2009; Li et al. 2011), which, additionally, can be seen as a reduction and an increase in the mass fraction of roots and shoots, respectively, according to Poorter et al. (2012). In some cases, the magnitude of this effect can be diminished partially—but not counterbalanced—as a result of the formation porous adventitious roots (see Malik et al. 2002), which help plants to alleviate the adverse effect of anaerobiosis by functionally replacing the original root system during flooding.

At this point, it is important to note that, immediately after flooding subsides, there will be an imbalance between the potential for transpiration (high) and for water uptake (low) as a consequence of the above-mentioned flood-induced changes in the shoot-to-root ratio. In this scenario, reductions in the shoot-to-root biomass ratio will be a logical response for restoring water homeostasis in the new condition of well drained soil. So, after flooding, root growth will be prioritized over shoot growth in order to re-establish the shoot-to-root ratio typical of plants growing under drained soil at the corresponding ontogenetic age (see shoot-to-root ratio re-establishment for *Lotus* spp. during recovery in Striker et al. 2012). In addition, after water recedes, the adventitious roots formed during flooding enter senescence, further leading to a need to promote new root growth. As a consequence, experiments including a recovery period but lacking in a quantification of root recovery will undoubtedly underestimate the real plant recovery. It should be noted that these ideas are still valid for B-type response plants (i.e., non-growing during floods) because after flooding both the shoots and the roots will resume their growth coordinately.

Root recovery can be assessed by both destructive and non-destructive sampling methods. The former method requires consecutive plant harvests during recovery (a larger number of plants will be required). At each harvest, measurements of root number, length, depth, and root branching, besides root mass, are recommended in order to evaluate more specific changes linked to the functioning of roots (see Nakai et al. 2009; Li et al. 2011). The second method monitors root system recovery using minirhizotrons, which are transparent plexiglass tubes inserted into the soil to provide a snapshot view of roots growing past an angled tube. Digital images of roots can be taken daily, and later processed and subjected to image analysis (as in Luo et al. 2011). This method has the advantage of allowing observation of the dynamics of root growth on the same plants throughout the entire experiment.

In summary, it is strongly suggested that researchers consider the inclusion of a recovery period after flooding as well as assessing root recovery within this period. Otherwise, by ignoring these issues, any conclusions drawn regarding plant tolerance to floods will be limited and possibly misleading, thereby defeating the purpose of the experiments.

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