# No escape? Costs and benefits of leaf de-submergence in the pasture grass *Chloris gayana* under different flooding regimes

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**Abstract.** Elongation-induced leaf emergence is one way for plants to deal with complete submergence by 'escaping' from water. This growth strategy is hypothesised to be more beneficial under single long-term submergence than under repeated short-term submergence events (i.e. fluctuating environment), as costs of repeated plant 'adjustment' would exceed the initial benefits of shoot elongation. To test this idea, 2-week-old plants of *Chloris gayana* Kunth. cv. Fine Cut (a submergence-tolerant cultivar first selected by a screening experiment) were grown for 4 weeks under (i) control conditions, (ii) two 1-week submergence cycles, or (iii) one 2-week submergence cycle. Additionally, a set of plants were placed below nettings to assess the cost of remaining forcedly submerged. Impeding leaves emergence through nettings did not compromise survival when submergence was 1-week long, but determined the death of all plants when extended to 2 weeks. Growth as affected by flooding regime revealed that under one 2-week submergence event, plants accumulated a 2.9-fold higher dry mass than when they experienced the same submergence duration in separate events along 1 week. The 'escape' strategy in the grass *C. gayana*, by which leaf contact with air is re-established, is essential for its survival, and it is more beneficial for plant growth under long-term submergence than under repeated short-term submergence cycles.

**Additional keywords:** *Chloris gayana* cultivars, flooding timing, leaf desiccation, leaf greenness, plant recovery, Rhodes grass, shoot elongation.

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## Introduction

As a result of global climate change, temperatures are increasing and this effect is expected to continue in the future, particularly the rise of both extremes, minimum and maximum temperatures (Hirabayashi *et al.* 2013). In temperate environments, this effect is predicted to result in less restrictive winters with shorter freezing periods affecting plant growth. These changes are allowing the introduction of subtropical grasses of high forage yield potential into temperate grasslands to increase forage production to be offered to livestock (Imaz *et al.* 2012, 2015). One of the most promising species in this new scenario is *Chloris gayana* Kunth., a subtropical grass being incorporated into lowland areas prone to flooding. Nevertheless, success in its introduction is erratic as

spring season flooding events could overlap with sowing. Evidence for differential tolerance among cultivars/accessions of *C. gayana* is available for abiotic stresses like salinity (Ribotta *et al.* 2013) and drought (Ponsens *et al.* 2010), but not for hypoxia due to plant submergence (only information for one cultivar is available, see Imaz *et al.* 2012). So, in a first experiment, we examined the variation in submergence tolerance of seven cultivars of *C. gayana* by evaluating growth responses and recovery ability during and after 2 weeks of complete submergence.

Another expected effect of global climate change is the increase in the frequency and intensity of flooding, which make these events even more unpredictable. Complete

submergence of the shoot is one of the most stressful scenarios that plants can confront in environments prone to flooding (Voesenek *et al.* 2006). Rapid shortage of oxygen and the shifting from aerobic to fermentative metabolism (Colmer and Voesenek 2009) can disrupt plant's energy and lead to biomass losses and eventually death of the submerged individuals. One of the main strategies to cope with this stress is an 'escape' strategy characterised by a fast elongation of the shoot organs upon submergence in order to rapidly restore leaf contact with the atmosphere (Bailey-Serres and Voesenek 2008; Pierik *et al.* 2009; Akman *et al.* 2012). Despite several studies having gained insight into plant responses and tolerance to submergence by using this strategy (reviewed by Bailey-Serres and Voesenek 2008; Striker 2012*a*; Striker *et al.* 2012), little is known about the responses of an 'escapist' species under different submergence regimes (only one study in four populations of the dicot *Rumex crispus* L., see Chen *et al.* 2011). So, in a second experiment, we investigated the effects of two flooding regimes – repeated submergence events vs a single submergence event – using the most promising cultivar identified in the first experiment. We hypothesised that the 'escape' strategy is more beneficial under long-term submergence than under repeated short-term submergence as the costs of recurrent plant 'adjustments' to cope with submergence would exceed the initial benefits. We used survival and dry mass production as fitness indicators (see van der Sman *et al.* 1993) in order to test the balance between costs and benefits under the different regimes. Also, we used an additional treatment where plants were not allowed to emerge from water by using tulle nettings below water. In this respect, only costs (no net benefits) of the leaf elongation-based 'escape' strategy were expected (see also Chen *et al.* 2011). So far, to our knowledge this is the first time experimental evidence is obtained for a grass species quantitatively demonstrating that (i) the costs of impeded leaf emergence (simulating deep water columns) are reflected in decreased survival along submergence time, and that (ii) the 'escape' strategy is clearly more beneficial for plant growth under long-term than under short-term repeated submergence for the same total time of submergence.

## Materials and methods

### Species description

*Chloris gayana* Kunth. (Rhodes grass) is a tropical grass native to Africa, and worldwide cultivated as one of the most important warm-season forage grasses in subtropical and tropical areas (Ponsens *et al.* 2010). Nowadays, this species is also being introduced in temperate areas as a consequence of increasing minimum temperatures (i.e. less restrictive winters) due to the effects of global warming (Imaz *et al.* 2012, 2015). It is used for direct grazing, to produce hay and silage, and also to stabilise sites with erosion problems. *C. gayana* is a stoloniferous and tufted, leafy perennial grass with ascending stems (0.5–1 m tall) and hairless leaves measuring 10–50 cm long. In particular, it is regarded as tolerant to soil salinity and drought (Dear *et al.* 2008) but it is also introduced in areas suffering from water excess of variable intensity, causing not only soil waterlogging (see Boschma *et al.* 2008) but also plant submergence (Imaz *et al.* 2012).

### Experiment 1. Variation in submergence tolerance among Chloris gayana *cultivars*

Seeds of *C. gayana* of seven commercial cultivars, namely Fine Cut, Pioneer, Tolga, Callide, Top Cut, Katambora and Epica, were germinated in an incubator (25°C) in polystyrene boxes containing absorbent white paper saturated with distilled water. After 2–3 days, germinated seeds were transplanted to 0.6 L plastic pots (three per pot) filled with sand and topsoil (1 : 1) from a natural grassland in the Flooding Pampa of Argentina (organic carbon 2.6%, community B3, see further details in work by Di Bella *et al.* 2016) and placed in a glasshouse at the Faculty of Agronomy at the University of Buenos Aires. Seedlings were left to grow for 14 days and subsequently thinned to one per pot.

Plants of each cultivar with three fully expanded leaves were subjected to two treatments for 14 days, following a fully randomised design with 10 replicates: (i) control (C): watered daily to field capacity, and (ii) complete submergence (CS): plants were submerged in clear water at a depth of 21 cm, which corresponded to a water column of 5–6 cm above the top of the plants simulating a shallow submergence (water column ~1.25 times the plant height). The photosynthetic photon flux density (PPFD) reaching plants under control conditions was $1120 \pm 140 \, \mu mol \, m^{-2} \, s^{-1}$, whereas for submerged plants (CS treatment), it was $530 \pm 60 \, \mu mol \, m^{-2} \, s^{-1}$ (LI-192 Underwater Quantum Sensor; Li-Cor Inc.). The latter was a light environment that allows for underwater photosynthesis in $C_4$ species (Colmer and Pedersen 2008).

After the submergence period, plants were allowed to grow under well drained conditions for 14 days to assess their recovery (Striker 2012*b*). Average daily temperature during the experiment in the glasshouse ranged between 20 and 27°C. The submergence duration, temperature and water depth applied are consistent with submergence scenarios that seedlings and young plants of this species can confront in lowland areas of the Flooding Pampas after being sown in spring (Striker *et al.* 2011; Imaz *et al.* 2012; Di Bella *et al.* 2016).

Total dry mass accumulation per plant (including shoots and roots) was measured at the end of submergence (day 14) and at the end of the recovery period (day 28). In all cases, plant material was weighed after oven drying for 72 h at 80°C. Treatment effect on dry mass was evaluated by two-way ANOVAs, with 'submergence' and 'cultivar' as the main factors. When significant interactions were detected, a subsequent Tukey's test was performed to determine the treatment effects.

### Experiment 2. Repeated short-term submergence vs single long-term submergence

Seeds of *C. gayana* of the cultivar Fine Cut, the most tolerant material from the previous experiment, were germinated and seedlings raised as described for experiment 1. Plants with three fully expanded leaves were subjected during 4 weeks to (i) control conditions: watered daily to field capacity (hereafter C +C+C+C), and two flooding regimes consisting of (ii) repeated short-term submergence in which plants were submerged twice during the first and third week of the experiment and remained under well drained conditions during the second and fourth week (hereafter S+C+S+C; C, control and S, submergence) and (iii) single long-term submergence by which plants were

submerged during the first 2 weeks and allowed to recover during the following 2 weeks (hereafter S+S+C+C). Therefore, in both flooding regimes, plants experienced the same time of submergence and recovery under well drained conditions (2 weeks each). A quick drainage of exceeding water was observed in pots (i.e. few hours) after each submergence event but when interpreting results, such fast drainage should not necessarily be expected to occur under field conditions. Plants were submerged in clear water at a depth of 19–21 cm, which corresponded to a water column similar to that used in the first experiment (5–6 cm water above the top of the seedlings). In the third week of the repeated short-term submergence treatment (i.e. second submergence event), plants were submerged in clear water at a depth of 30–33 cm, which corresponded to a water column of 7–9 cm above the top of the plants (water column ~1.25 times the plant height, thus simulating a shallow submergence). To examine the costs of not allowing plants to exhibit the 'escape' strategy, tulle nettings were used to prevent leaf emergence, placing them 2 cm below water in additional containers with plants under both flooding regimes. Also, tulle nettings were placed in containers with control plants to verify that plant growth was not affected by the netting treatment (i.e. nettings reduced irradiance only by 5%). The photosynthetic photon flux density (PPFD) that reached plants in contact with atmospheric air (control treatment) was $1240 \pm 180\,\mu mol\,m^{-2}\,s^{-1}$ without nettings and $1160 \pm 150\,\mu mol\,m^{-2}\,s^{-1}$ below nettings. Under submergence treatments the PPFD reaching plants was $544 \pm 20\,\mu mol\,m^{-2}\,s^{-1}$ without nettings and $514 \pm 16\,\mu mol\,m^{-2}\,s^{-1}$ with nettings (values were similar between the first week in both submergence treatments and the third week of submergence despite the slight differences in the depth of water columns above plants: 5–6 vs 7–9 cm) (LI-192 Underwater Quantum Sensor; Li-Cor Inc.). Average daily temperature during the experiment in the glasshouse ranged between 19 and 28°C.

Plants were harvested at the beginning of the treatments (day 0) and at the end of every week (days 7, 14, 21 and 28) with shoots being separated from roots to examine the progression of dry mass accumulation. Leaf greenness was measured in fully expanded young leaves using a portable chlorophyll meter (Model SPAD-502, Minolta) at the same harvest times to infer the damage suffered by the photosynthetic apparatus due to submergence. The relative water content of leaves (RWC) was measured according to work by Čatský (1960) in leaves similar to those used to measure greenness, to deduce potential leaf desiccation after submergence and its impact on plant recovery. First, 5 cm long leaf blade samples (2.5 cm at each side of the centre of the leaf blade) were cut with a razor blade 2–3 h after water was removed in the submergence treatments, and immediately weighed to obtain their FW. Second, samples were incubated for 12 h in tightly-closed tubes containing distilled water to obtain their turgid weight (TW). Third, the leaves were oven-dried at 70°C for 48 h to obtain their DW (the weights of leaves were later added to the shoot compartment of the corresponding plants). Finally, the RWC of leaves was obtained as: $((FW - DW)/(TW - DW)) \times 100$.

Plant survival was affected only by the presence of netting impeding leaf de-submergence, which was assessed through survival analyses ($\chi^2$: chi-square test). Values of dry mass, leaf greenness and relative water content at the end of experiment were examined by two-way ANOVAs, with 'submergence regime', and 'netting' as main factors. When significant interactions were detected, subsequent Tukey's tests were conducted to determine treatment effects. Additional one-way ANOVA tests were performed to examine changes in the above-mentioned variables when comparing values among control, short-term and long-term submergence treatments separately for each netting condition.

## Results

### Submergence tolerance varied among Chloris gayana cultivars

At the end of the submergence period, dry mass of submerged plants was similar among cultivars (16.7–23.5 mg plant$^{-1}$; Fig. 1*a*), but 38–57% lower than their respective controls ('submergence' effect in Table 1; Fig. 1*a*). When under control conditions, plants of the cultivars Fine Cut, Pioneer, Callide and Epica attained the highest values of dry mass
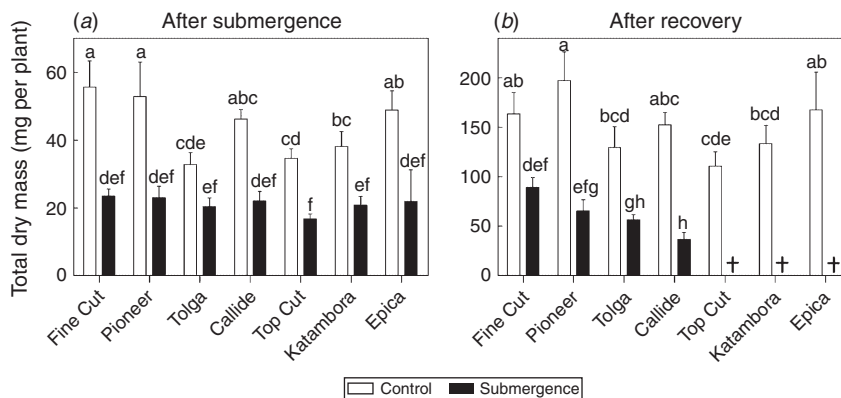


**Fig. 1.** Total dry mass (shoots and roots) of 2-week-old plants of seven cultivars of *Chloris gayana* subjected to control and submergence conditions for 14 days (*a*), and after a subsequent 14 days period of recovery at well drained conditions (*b*). Different letters indicate significant differences among cultivars/treatments within each period (submergence and recovery). Values are means ± s.e. of 10 replicates; † indicates no surviving plants. Note the different scales between left and right panels.

**Table 1.    *F*- and *P*-values for the two-way ANOVA (factors: cultivar, treatment and cultivar × treatment) for dry mass responses of Experiment 1[A]**

Main effects and interactions were considered significant at $P < 0.05$. Analyses were performed separately for the submergence and the recovery periods

| Period | Cultivar | | Submergence treatment | | Cultivar × treatment | |
|---|---|---|---|---|---|---|
| | *F*-value | *P*-value | *F*-value | *P*-value | *F*-value | *P*-value |
| After submergence | 2.27 | 0.041 | 70.12 | <0.001 | 1.01 | 0.428 |
| After recovery | 5.25 | <0.001 | 153.9 | <0.001 | 2.87 | 0.038 |

[A]Degrees of freedom for each source of variation were: 6 (cultivar), 1 (treatment), 6 (cultivar × treatment) and 126 (error).

('cultivar' effect in Table 1). We noted that plants of all cultivars were able to emerge from water when submerged (water column 5–6 cm above plants) displaying the leaf elongation-based 'escape' strategy (see Fig. S1, available as Supplementary Material to this paper).

Two weeks after submergence, the assessment of plant recovery revealed that three out of the seven cultivars had perished, namely Top Cut, Katambora and Epica (all plants of these cultivars had died; Fig. 1*b*; significant 'cultivar' × 'submergence' interaction in Table 1). In contrast, among the surviving cultivars, Fine Cut and Pioneer showed the highest dry mass accumulation both at control and after recovering from submergence, with the former one showing slightly better performance (Fig. 1*b*). So, for a second experiment, we decided to use the cultivar Fine Cut as a promising tolerant material to test the effects of different submergence regimes.

### Costs of not allowing leaf-emergence under two regimes of submergence

An increased leaf lengthening under submergence was registered 1 week after submergence started: leaf blade and leaf sheath of submerged plants (with and without nettings) were 58% larger with respect to control plants (leaf blade: $14.5 \pm 0.75$ cm vs $9.15 \pm 0.62$ cm; leaf sheath: $6.58 \pm 0.23$ cm vs $4.16 \pm 0.45$ cm; $P < 0.05$). At the end of the second week of submergence, leaf length of the plants that remained underwater due to the nettings was similar to that of the previous week (leaf blade: $16.5 \pm 1.3$ cm; leaf sheath $6.1 \pm 0.44$ cm), whereas those that emerged above water (without nettings) were able to continue elongating their leaves (leaf blade: $23.0 \pm 1.2$ cm; leaf sheath $9.1 \pm 0.62$ cm).

Consequently, when plants were allowed to de-submerge their leaves (treatment without nettings), the result under both regimes, repeated short-term submergence and single long-term submergence, was 100% survival (Fig. 2*a, b*). In contrast, when plants forcedly remained under complete submergence due to the use of nettings, survival was compromised depending on the submergence regime ($\chi^2$: 159.8; $P < 0.001$). Under repeated short-term submergence, plants started perishing after a second event of submergence (day 21) dropping to 50% survival plants by the end of the experiment (day 28; Fig. 2*a*). Under single long-term submergence, 20% of the plants were visibly dead immediately after 2 weeks submerged below the netting (Fig. 2*b*), and shortly afterwards, all the remaining plants showed acute tissue desiccation without ability to resume growth even after allowing for 2 weeks recovery (Fig. 2*a*). As expected, all control plants showed 100% survival along the experiment.
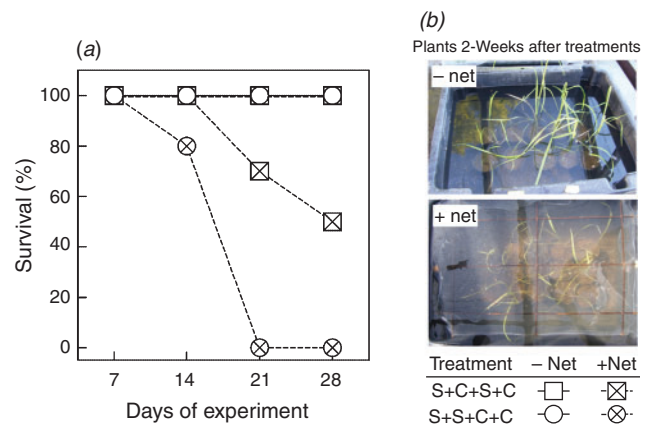


**Fig. 2.**   Survival (*a*) of 2-week-old plants of *Chloris gayana* cv. Fine Cut subjected to repeated 1-week submergence (squares) and single 2-week submergence (circles) in combination with the presence (crosses) or absence (open symbols) of nettings at 2 cm below water to prevent leaf de-submergence. Emerged plants as a result of the leaf-elongation-based 'escape' strategy (*b*) when previously submerged without netting (right above) and (forcedly) submerged plants below water due to the netting (right below) 2 weeks after imposing submergence. Survival of non-submerged plants (not shown in the figure for clarity) was 100% throughout the experiment. Abbreviations: C, control; S, submergence.

### Higher benefits for plant growth under single long-term submergence than under repeated short-term submergence

At the end of the experiment (day 28), shoot and root dry mass accumulation were 3-fold and 2.4-fold higher under single long-term submergence (one event, 2 weeks duration) than under repeated short-term submergence (two events, 1 week duration each), indicating that performance by using the 'escape' strategy was better under a non-fluctuating environment (Table 2). The benefit in growth was already apparent at day 21 (1.9- and 1.8-fold higher shoot and root dry mass under long-term submergence than under repeated short-term submergence) (Fig. 3*a, c*). As expected, plants growing under non-stressful conditions (i.e. control treatment) reached shoot and root dry masses between 2.5- and 7-fold and 2.4- and 6-fold higher than submerged ones (compared with long-term and short-term submerged regimes respectively). Importantly, the use of nettings did not affect plant mass accumulation of non-submerged plants, which reached similar values with and without nettings ($P > 0.32$ for shoot and roots; Fig. 3), indicating that nettings only impeded leaf emergence without affecting plant growth.

**Table 2.   *F*- and *P*-values for the two-way ANOVAs (factors: submergence regime (SR), netting treatment and SR × netting) for final values of shoot and root dry mass, leaf greenness, and leaf relative water content of Experiment 2[A]**

| Variable | Submergence regime (SR) | | Netting treatment | | SR × netting | |
|---|---|---|---|---|---|---|
| | *F*-value | *P*-value | *F*-value | *P*-value | *F*-value | *P*-value |
| Shoot dry mass | 65.7 | <0.001 | 7.27 | 0.0096 | 2.86 | 0.040 |
| Root dry mass | 60.3 | <0.001 | 9.67 | 0.0031 | 2.91 | 0.036 |
| Leaf greenness | 21.7 | <0.001 | 42.5 | <0.001 | 12.3 | <0.001 |
| Relative water content | 130.2 | <0.001 | 276.8 | <0.001 | 81.5 | <0.001 |

[A]Degrees of freedom for each source of variation were: 2 (submergence regime, SR), 1 (netting treatment), 2 (SR × netting) and 54 (error).
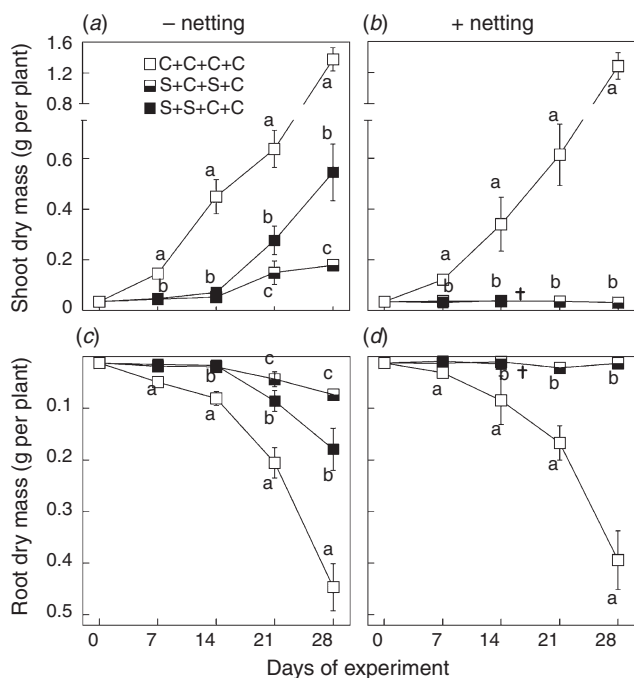


**Fig. 3.** Shoot (*a*, *b*) and root (*b*, *c*) dry mass accumulation of 2-week-old plants of *Chloris gayana* cv. Fine Cut subjected to control (open squares), repeated 1-week submergence (half-closed squares) and single 2-week submergence (closed squares). Dry mass of plants growing in absence (*a*, *c*) and presence (*b*, *d*) of nettings are shown. Abbreviations: C, control; S, submergence. Note that plant dry mass at 21 days and 28 days under repeated 1-week submergence when under the netting reflect the growth of the survival plants (see Fig. 2). Values are means ± s.e. of 10 replicates, † indicates no surviving plants when under single long 2-week submergence plus netting. Different letters indicate significant differences among treatments within each 'netting' condition and harvest date.



**Fig. 4.** Leaf greenness (*a*, *b*) and leaf relative water content (RWC; *c*, *d*) of 2-week-old plants of *Chloris gayana* cv. Fine Cut subjected to control (open squares), repeated 1-week submergence (half-closed squares) and single 2-week submergence (closed squares). Values for plants growing in absence (*a*, *c*) and presence (*b*, *d*) of nettings are shown. Abbreviations: C, control; S, submergence. Leaf tissue samples for RWC measurements were taken 2–3 h after de-submergence. Values are means ± s.e. of 10 replicates, † indicates no surviving plants when under single long 2-week submergence plus netting. Different letters indicate significant differences among treatments within each 'netting' condition and measurement date.

## Plant performance was related to the ability to retain chlorophyll and to recover leaf water status after submergence

Leaf greenness was reduced by almost 30% (compared with controls) at the end of the first week in submerged plants without nettings (Fig. 4*a*). Plants that remained submerged for another week (single long-term submergence) reduced this parameter even further (42% with respect to the controls), whereas in those de-submerged after the first week, this parameter was maintained, noticing a slight trend towards recovery. We noted that after long-term submergence, leaf greenness of plants
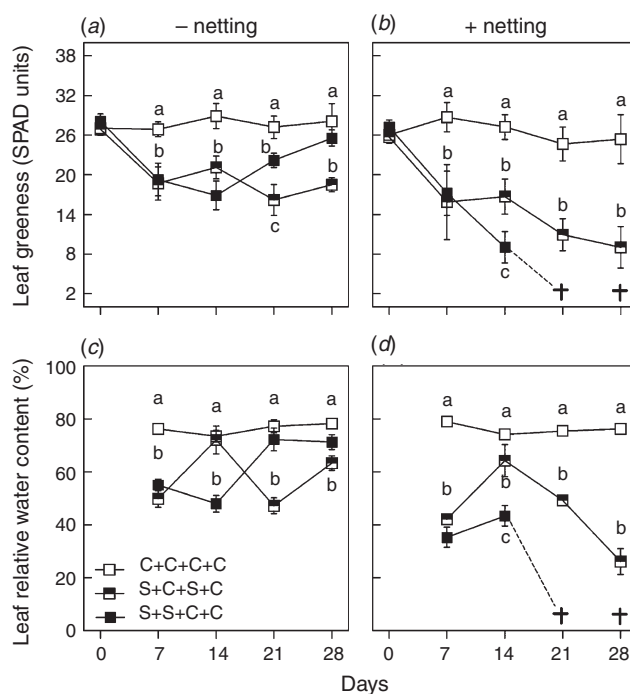
was progressively recovered until reaching values similar to controls by the end of the experiment (see day 28 in Fig. 4*a*; Table 2). In contrast, under repeated short-term submergence, plants always showed lower values for this parameter (reductions of 34–40% with respect to controls; Fig. 4*a*).

In plants that remained below water due to the use of nettings, leaf chlorophyll degradation was accelerated as indicated by the higher reductions observed in greenness (Fig. 4*b*; 'submergence' × 'netting' interaction in Table 2). After 14 days of submergence, greenness dropped by 67% with respect to controls in plants where leaves de-submergence was prevented; following this period, plants submerged by nettings

started wilting (Fig. 4*d*) and dying (Fig. 2*a*). In the case of plants subjected to repeated short-term submergence, the values registered for leaf greenness of remaining alive plants (half the plants survived by day 28) did not recover between submergence periods, and further reductions of up to 65% were observed compared with controls by the end of the experiment (Fig. 4*b*). The lack of leaf greenness recovery was in line with the negligible growth of those plants, which was expected from an injured photosynthetic capacity.

The dynamics of the relative water content (RWC) of leaf tissues over time reflected the plants' ability to deal with desiccation after submergence. This response differed if submerged plants were allowed to de-submerge their leaves or not (significant 'submergence' × 'netting' interaction in Table 2). Plants submerged by 1 week and allowed to emerge from water (without nettings) showed 65–72% lower RWC relative to controls when sampled few hours (2–3 h) after de-submergence, indicating rapid leaf desiccation. Under repeated short-term submergence, newly formed leaves were able to fully recover the leaf water status after 1 week under well drained conditions during the first cycle of submergence-recovery, but they recovered only partially after the second cycle, where the RWC dropped by 20% with respect to controls (Fig. 4*c*). Under single long-term submergence, RWC fully recovered in new leaves, reaching values similar to controls after 1 week of de-submergence, remaining unchanged until the end of the experiment (Fig. 4*c*). In the case of plants submerged by nettings, under repeated short-term submergence, the RWC almost fully recovered 1 week after de-submergence. However, after the second submergence cycle, leaf tissue started desiccating progressively to reach 66% lower RWC compared with control values in the remaining alive plants at the end of the experiment (Fig. 4*d*).

## Discussion

The present research makes three main contributions to current knowledge: first, there is variation in the tolerance to shallow submergence in clear water of *C. gayana* cultivars where, despite the fact that all are able to de-submerge leaves 'escaping' from water during submergence, some materials are able to resume growth after water subsides (Fine Cut, Pioneer, Tolga and Callide) and others are not and perish (Top Cut, Katambora and Epica). This is one of the first reports on cultivars' variation in submergence tolerance of a forage species. Second, the prevention of leaf emergence in this 'escapist' species progressively impacts on plant survival along the submergence time, which indicates that de-submergence is essential when water rises above soil. Third, in the most tolerant cultivar of *C. gayana*, namely Fine Cut, the flooding regime (i.e. timing and length of submergence) strongly affects plant dry mass accumulation (but not survival). For the same total time of submergence of 2 weeks, a single long-term submergence event (S+S+C+C) allowed plants to accumulate 3- and 2.4-fold higher shoot and root dry mass, respectively, than two short-term submergence events (S+C+S+C). This strongly suggests that the costs incurred in developing the 'escape strategy' are high when the environment changes rapidly before plants can take benefit from the induced plastic

adjustments (e.g. leaf elongation to allow de-submergence) (see also Chen *et al.* 2011 for work on the dicot *Rumex palustris*).

Here we reported for the first time, the existence of variation in responses to submergence of *C. gayana* cultivars (Fig. 1), which paves the way to consider the development of a, currently non-existent, breeding program for submergence tolerance in this species (see Malkin and Waisel 1986; for an example of mass selection for 'salt resistance' in *C. gayana*). Moreover, our results suggest that the efforts should be focussed on investigating the traits and mechanisms underlying the differential ability of these materials to recover from submergence rather than on the responses during submergence *per se* (Striker 2012*a*; Striker and Colmer 2017 for forage legumes).

In this case, the high constitutive root aerenchyma reported for different genetic materials of this species (>30% of root aerenchyma, Taleisnik *et al.* 1999; Imaz *et al.* 2012) does not appear to limit root aeration, so potential differences in aerenchyma generation among cultivars are not likely to reflect a differential plant performance as indicated by the similar dry mass accumulated in all cultivars at the end of the submergence period (Fig. 1*a*). In contrast, assessment of plant responses soon after water subsides like the regulation of plant water status, high ability to deal with reactive oxygen species (ROS), delayed senescence and the potential links thereof are aspects that should be investigated. Regarding ROS, a recent work using submergence-tolerant and sensitive ecotypes of the model grass *Brachypodium distachyon* showed that the ability to deal with oxidative stress is a major tolerance factor as revealed by transcriptomic analysis (Rivera-Contreras *et al.* 2016).

The use of nettings demonstrated that the survival strategy of this species to deal with long submergence periods (>1 week) relies on escaping from water. The first evidence of this was that leaf elongation during the first week was the same in plants allowed to emerge as in those that remained underwater due to nettings. This response might be attributed to an increase in ethylene concentration in submerged tissues as a first step of a well-known signalling pathway during hypoxia, by which abscisic acid is repressed and, thereby gibberellins increase promoting leaf lengthening (Sasidharan and Voesenek 2015; Voesenek and Bailey-Serres 2015). Later, as leaves did not emerge, leaf elongation stopped as observed by the end of the second week. One possible cause is that leaves could not sustain further elongation due to the lack of energy/ATP either related to exhausted reserves or limited current carbon assimilation (Setter and Laureles 1996; Striker *et al.* 2011; and review by Loreti *et al.* 2016). Another potential explanation is associated with the triggering of leaf senescence due to continued accumulation of ethylene that could not be released to the atmosphere as leaves were not allowed to emerge. Out of the two mentioned (non-exclusive) causes, the second one is supported by a steepest drop in leaf greenness for plants initially submerged below nettings after the first week (17 vs 9 SPAD units at days 7 and 14, respectively; Fig. 4*d*). The first cause is less likely, considering that submergence was shallow in clear water and a considerable flux of photons reached submerged leaves, thereby some level of underwater photosynthesis could be expected (Colmer and Pedersen 2008), even more as leaves of this species are able to retain leaf gas films upon submergence (G Striker, pers. obs.). Nevertheless, we cannot rule out the first

explanation as the potential level for underwater photosynthesis of this species has not been tested yet.

The benefits of escaping from water in terms of dry mass accumulation were clear when plants were subjected to single long-term submergence compared with repeated short-term submergence. Under a fluctuating environment where plants were subjected to short submergence events alternated with periods of drained soil, the costs overweighed the benefits of escaping due to enhanced leaf elongation as 3-fold lower dry mass per plant was attained. Rapid leaf lengthening, as it occurred during submergence, often derives in biomechanically weaker leaves with thin cuticles (Mommer and Visser 2005), which might also have problems of decreased hydraulic conductivity in the leaf sheath as seen in rice (Setter *et al*. 2010). So, soon after water subsides, these leaves start desiccating (Fig. 4c), eventually dying, and consequently constituting a cost for plants, as they did not have enough time to get benefits from such leaf elongation response, which allowed improving gas exchange and photosynthesis during de-submergence (Voesenek *et al*. 2004; Chen *et al*. 2011). For future carbon fixation, previously submerged plants mostly depend on photosynthesis of leaves formed during the post-submergence (Chen *et al*. 2011), thereby the high leaf turnover due to the death of flooding-acclimated leaves under repeated short-term submergence implies a high loss of previously invested carbon during submergence, either from reserves (Striker *et al*. 2011) or underwater photosynthesis (Colmer and Pedersen 2008).

Major differences in dry mass accumulation were observed during plant recovery from submergence rather than during submergence *per se* (Figs 1, 3). In this experiment, plant recovery was likely related to the ability to retain (and later to recover) leaf nitrogen (measured here as greenness) contributing to the potential for carbon fixation. Under long-term submergence, greenness in newly formed leaves was progressively recovered until the end of the experiment to reach values similar to controls. In contrast, under repeated short-term submergence, greenness was further reduced during a second submergence cycle, which concurs with the very low dry mass accumulation during the last week of the experiment (Figs 3, 4) as expected from a nitrogen-limited photosynthesis (Malik *et al*. 2002). So, a good nitrogen status of plants appears to be essential when thinking of a quick plant recovery. In this respect, the balance of nitrogen during (e.g. losses via denitrification, up to 10–15% according to Fillery and Vlek 1982) and after soil flooding (e.g. inputs via fertilisation in work by Gautam *et al*. 2017; for rice; fixation of atmospheric nitrogen by legumes in Striker and Colmer 2017) should be considered as well as the capability of roots after submergence for nutrient uptake.

We conclude that *C. gayana* has genotypic variability in its response to complete submergence, which is critical to think of future breeding purposes aimed at improving its submergence tolerance. The species presents a leaf-elongation based 'escape' strategy to deal with complete submergence as seen in the screened cultivars. This response allows plants to emerge leaves above water to facilitate tissue aeration as it has considerable amounts of constitutive aerenchyma in roots (>30%; Imaz *et al*. 2012), which is further increased under submergence (Imaz *et al*. 2012). The alternative strategy of remaining quiescent until water recedes, more appropriate to deal with deep submergence scenarios (Colmer and Voesenek 2009; Striker 2012b), was not found in the cultivars studied. Under fluctuating environments, where cycles of soil water excess rapidly alternate with drained soil conditions, the performance of this grass species (cv. Fine Cut) is highly reduced. In these cases, the costs of the repeated plant 'adjustment' to each scenario overweigh the benefits of the initial development of the 'escape' strategy in terms of growth and dry mass accumulation. Therefore, the 'escape' strategy in *C. gayana* by which leaf contact with air is re-established is essential for its survival, and it is more beneficial for plant growth under long-term submergence than under repeated short-term submergence cycles.

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