10.1071/FP23184

Functional Plant Biology

Supplementary Material

Boosting underwater germination in *Echinochloa colona* seeds: the impact of high amplitude alternating temperatures and potassium nitrate osmopriming

Juliana Echeverry Holguín[A,B], *María Crepy*[C], *Gustavo G. Striker*[A,B,D], *and Federico P. O. Mollard*[A,B,*]

[A]Departamento de Biología Aplicada y Alimentos, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina.

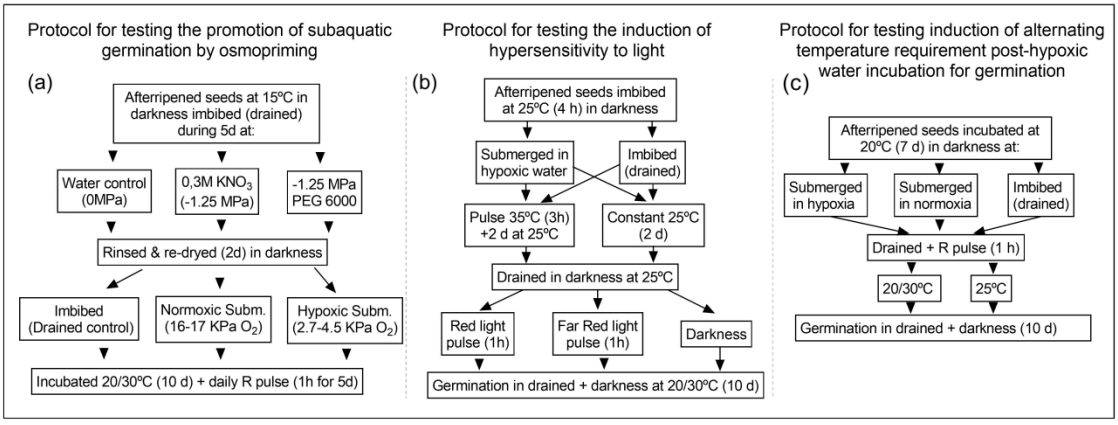
[B]IFEVA, Universidad de Buenos Aires-CONICET, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina.

[C]EEA Concepción del Uruguay-INTA, Ruta 39, km 143.5, Concepción del Uruguay, Entre Ríos CP 3260, Argentina.

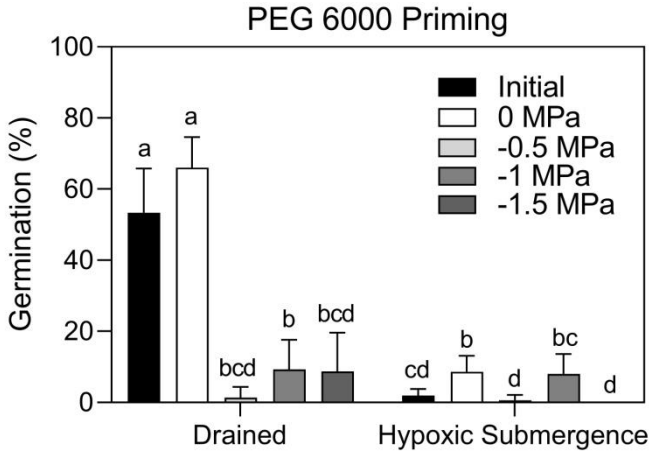
[D]School of Agriculture and Environment, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

[*]Correspondence to: Federico P. O. Mollard Departamento de Biología Aplicada y Alimentos, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina
Email: fmollard@agro.uba.ar

Supplementary Figure S1. Overview of protocols followed in experiments 2, 4 and 5 with *Echinochloa colona* seeds**.** (a) Flow diagram of experiment 2: seeds were incubated at 15ºC in darkness under the following pre-treatments: dH_2O control (0 MPa), KNO_3 or PEG 6000 solution during 5d. Seeds were rinsed and re-dried before subjected to treatments under an AT regime (20/30°C, 9/15 h d^{-1}) and daily red-light pulses: submerged under hypoxic dH_2O, submerged under atmosphere-equilibrated dH_2O or imbibed on filter paper (drained control). (b) Flow diagram of experiment 3: seeds were incubated under hypoxic dH_2O (pO_2 = 2.8-5 kPa) or imbibed on filter paper (drained control) at 25ºC for 2d. Half of the replicates were subjected to a 35ºC pulse (3h). At the end of the second day all seeds were drained and exposed to either a Red-light pulse (Rp, 1h), a Far-Red light pulse (FRp, 1h) or kept in darkness. Then seeds were transferred to a 20/30°C (9/15 h d^{-1}) regime to evaluate germination. (c) Flow diagram of experiment 4: shallow dormant seeds were incubated at 20ºC in darkness under one of the following treatments: submerged under hypoxic dH_2O, submerged under air equilibrated dH_2O or imbibed on wet filter paper (drained control). After 7d seeds were drained, subjected to a Red-light pulse, and put to germinate during 10d under either an AT (20/30°C, 9/15 h d^{-1}) or constant temperature (25ºC) regime.



Supplementary Figure S2. *Echinochloa colona* seeds secondary dormancy induction by osmotic priming under a range of PEG 6000 solutions with different water potential. Seeds were incubated at 15ºC in darkness under one of the following pre-treatments: imbibed dH2O control (0 MPa) or imbibed in PEG 6000 solutions during 5d. Seeds were rinsed and re-dried before subjected to the following germination treatments under an AT regime (20/30°C, 9/15 h d-1) and red-light daily pulses: submerged under hypoxic dH2O or imbibed on wet filter paper (drained control). Germination percentages after 10d are presented as means ± SE (N=5). Bars not sharing the same letter are significantly different ($p < 0.05$).



Supplementary Figure S3. Testing the promotion by a Far Red light pulse of *Echinochloa colona* underwater seed germination through the VLFR mode of action of phytochromes. Seeds were incubated under submergence or hypoxic submergence conditions. Seeds were incubated during 1d at 25ºC in darkness and then subjected to a AT regime (35/25°C, 9/15 h d-1) during 2d to promote VLFR. At the end of the third day seeds were exposed to either a Red-light pulse (Rp, 1h), a Far-Red light pulse (FRp, 1h) or kept in constant darkness. Then seeds were transferred to a 20/30°C (9/15 h d-1) regime. All manipulations were done in constant darkness. Germination percentages after 10d are presented as means ± SE (N=4). Bars not sharing the same letter are significantly different ($p < 0.05$).