

OVERCOMING SEED DORMANCY OF JUNGLERICE (*Echinochloa colona*)

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ABSTRACT

Junglerice (*Echinochloa colona* L. Link) seed dormancy can persist 8 months after harvest and, once overcome, seeds can germinate over a long period of time. Light or high temperatures could overcome seed dormancy, while the effects of seed coat softening and nitrate addition are not well known. This study aimed to determine the germinative response, persistence and treatments to overcome seed dormancy of junglerice. Seed samples were taken from a monoculture crop (soybean, S1) and a 3-yr rotation crop (wheat/soybean-corn-soybean, S2). Every 3 wk for 30 wk, hot-water washed (WW) seeds were evaluated. After 33 wk of storage, the effects of potassium nitrate (KNO₃), dehulling (DE), WW, previous water immersion (IM) and exogenous gibberellic acid (GA) were evaluated in three different experiments (pre-germinative tests with KNO₃, DE and WW; KNO₃ and IM; and GA). The results showed that seed dormancy persisted until 21 wk. From 21 to 30 wk, germination response increased by 25 and 59 % on WW-treated seeds from S1 and S2, respectively. After 33 wk of storage, only KNO₃ was significantly higher ($p < 0.05$), with 88 and 126 % increases in seed germination in S1 and S2, respectively. These results indicate that junglerice seed dormancy is not related to glumes. Hot water improves the germinative response probably due to an acceleration in post-harvest maturation, while KNO₃ is a positive regulator to overcome seed dormancy.

Keywords: Dehulling, hot-water washing, KNO₃, water immersion.

INTRODUCTION

The genus *Echinochloa* Beauv. comprises about 50 species, being *E. colona* (L.) Link one of the most serious weeds in several crops (Bajwa et al., 2015). It emerges close to the beginning of the spring (Picapietra et al., 2020), and it could severely harm crop yield due to its ability to grow and produce 4000 to 10000 seeds plant⁻¹ at different

growth densities (Matloob and Chauhan, 2021). Seeds can germinate at superficial soil strata, reaching maximum germination at soil surface, and declining as burial depth increases up to 6 cm (Peerzada et al., 2016). Some *E. colona* accessions are positively photoblastic (germination stimulated by light), but others germinate in darkness (Mutti et al., 2019).

Soil seed persistence can be affected by time and

burial depth (Long et al., 2015). Seed persistence decreases by 19 % under 2-cm burial depth over a 6-month period, but seeds buried at 10 cm can persist longer (Peerzada et al., 2016). However, seed persistence would not be compromised due to the high number seeds plant⁻¹ produced or viability for more than 1 yr (Peerzada et al., 2016). The soil seedbank can reach an average of 24750 seeds m⁻² and its persistence can be relatively long (up to 6 yr) depending on burial depth (Shabbir et al., 2019).

Different seed dormancy periods have been reported for *E. colona* seeds. Some seed populations have optimum germinative response 2 months after ripening, with sufficient soil moisture and 25 °C incubation temperature, while other populations show that seed dormancy is lost within 4 months of soil burial (Tahir and Roma-Burgos, 2021). Other studies have shown that seed dormancy could be extended up to 7 months after harvest, while a secondary dormancy may be induced after water immersion (Peerzada et al., 2016; Bazzigalupi and Picapietra, 2015).

Several treatments have been tested to break *E. colona* seed dormancy. In this sense, cold stratification, and gibberellins addition have proven ineffective to overcome dormancy of these seeds, whereas high temperature, acid scarification, and puncturing (with a scalpel) have been effective in labor-intensive production (Tahir and Roma-Burgos, 2021). In addition, a study conducted by Kim et al. (2016) showed that immersing on ethanol induces seed germination. However, sequential light-condition test is recommended because *Echinochloa* seed accessions can be light-requiring (photodormancy) or dark-requiring (skotodormancy) (Tahir and Roma-Burgos, 2021).

Nitrate has been evaluated to break seed dormancy and promote subsequent germination in numerous plant species. Due to this, several researchers have used potassium nitrate (KNO₃) as a treatment to relieve seed dormancy (Shu et al., 2016; Arc et al., 2013). The use of hot water as a treatment to promote seed germination has also been used (Flores Romayna et al., 2020). A study conducted by Bazzigalupi and Picapietra (2015) showed that, after washing with water at 70 °C for 1.5 min, 50 % of seeds germinated 20 d after sowing.

Seed persistence allows weed seeds to disperse through time and survive, and germination is the success of the soil seed bank, depending on dormancy (Long et al., 2015). Knowing how seed dormancy is both modulated and overcome is of great importance to understand the weed growth process and its impacts on the crop.

Understanding *E. colona* seed germination,

particularly considering that each accession could have different requirements to overcome seed dormancy, is a matter of importance. Therefore, the aim of this study was to determine the germinative response, persistence and treatments to overcome dormancy of local populations of *E. colona*. Due to the disparity of results about the effectiveness of hot water in seed dormancy, we hypothesized that (i) hot water could be effective immediately after seed collection; (ii) the use of potassium nitrate, hot water washing, dehulling, water immersion, and gibberellic acid, alone or in combination, will increase the stored seed germination rate.

MATERIALS AND METHODS

Experiments were conducted in the National Institute of Agricultural Technology (INTA), Pergamino (33°53' S, 60°34' W), Buenos Aires province, Argentina. *E. colona* seed samples from soybean (*Glycine max* [L.] Merr.) monoculture field (S1) and a typically 3-yr rotation field (S2, wheat (*Triticum aestivum* L.)/soybean-corn (*Zea mays* L.)-soybean) were analyzed. On 16 March 2015, mature plants with seeds easily detached from the panicle, were harvested. Eighty panicles from randomly selected plants were taken from each field.

Seeds were cleaned by removing impurities and vain seeds, and then stored in a dark dry chamber at 20 °C. Viability was determined by the tetrazolium test, with four replicates of 50 seeds each. The seeds were water-imbibed by placing them between moistened sheets of paper at 7 °C for 18 h, and then placed in a 2,3,5-triphenyltetrazolium chloride (TTZ) solution (0.2% g l⁻¹) at 34 °C for 24 h. The evaluation was carried out by visualizing embryo staining (ISTA, 2015).

Up to treatment application, 20 seeds with a 30 g m⁻² paper substrate moistened with 15-ml water were placed in covered plastic boxes. Subsequently, boxes were placed in a germination chamber with an alternating temperature of 30/20 °C and 12 h light. Each treatment was replicated three times (60 seeds per treatment) for each sample.

Since there are no previous studies in *E. colona*, the protocol established for *E. crus-galli* (L.) P. Beauv. in accordance with ISTA standards (ISTA, 2015) was used. The number of germinated seeds was registered 21 d after sowing.

Dormancy experiment

Two days after seed collection, 120 *E. colona* seeds from each stored sample were extracted. One half was hot-water washed (WW) with

rinsing water at 70 °C for 1.5 min (Bazzigalupi and Picapietra, 2015), and the other half was not treated. The procedure was repeated every 3 wk for a period of 30 wk: 18 March, 8 April, 29 April, 20 May, 10 June, 7 July, 22 July, 14 August, 2 September, 23 September, and 14 October 2015.

After 33 wk of storage, WW, potassium nitrate solution (KNO₃), gibberellic acid (GA), dehulling (DE) and water immersion (IM) were evaluated through three different experiments (pre-germinative tests with KNO₃, DE and WW; KNO₃ and IM; and GA) (Table 1).

Pre-germinative test with KNO₃, DE and WW

The WW was carried out as described previously. KNO₃ was used as breaking dormancy treatment by wetting substrate. For this purpose, 15-ml water and the same volume of a solution of 0.2 % g l⁻¹ KNO₃ were used. The DE consisted of the manual removal of the lemma and palea from the seeds using a 40X binocular magnifying glass and tweezers.

A completely randomized design with factorial arrangement was used; the three factors, KNO₃, DE and WW, were included in two levels each: (0) negative control and (1) KNO₃, DE and WW testing. Interactions between factors were determined with a total number of eight treatments: KNO₃, DE, WW, KNO₃ ' DE, KNO₃ ' WW, WW ' DE, KNO₃ ' DE ' WW, and the untreated control.

Pre-germinative test with KNO₃ and IM

Application of KNO₃ solution was carried out as described previously. The IM consisted of placing seeds in a container with water for 48 h at 7 °C, immediately before sowing.

A completely randomized design with factorial arrangement was used; the factors KNO₃ and IM were included in two levels each: (0) negative control and (1) KNO₃ and IM testing. In this experiment, four total treatments were determined: KNO₃, INM, KNO₃ ' INM, and the untreated control.

Pre-germinative test with GA

The use of GA was tested as breaking dormancy treatment by wetting substrate. For this purpose, 15-ml water and the same volume of a solution of gibberellic acid at 0.8 % g l⁻¹ were used

A simple completely randomized design was postulated, where the 'treatment' effect were two levels of use of GA: (0) negative control and (1) GA testing.

Statistical analysis

With the number of germinated seeds (per box) obtained 21 d after sown (DAS), percentage of germinated seeds was calculated, considering the viable sown seeds (Eq. 1):

$$G(\%) = \frac{g_i}{g_t} \cdot v \cdot 100 \tag{Eq. 1}$$

Table 1. Treatments applied (Id. Tr) in each experiment (Id. Experiment): untreated seeds (UN), hot-water washing (WW), potassium nitrate (KNO), dehulling (DE), water immersion (IM), and exogenous gibberellic acid (GA). Treatments of experiment #1 were applied since seed collection, every 3 wk during 30 wk. Treatments of experiments #2, #3 and #4 were applied only once at 33 wk after seed collection.

Id. Experiment	Id. Tr	UN	WW	KNO	DE	IM	GA
#1 Dormancy experiment	T1	○					
	T2		○				
#2 Pre-germinative test with KNO ₃ , DE and WW	T1	○					
	T2				○		
	T3		○				
	T4		○		○		
	T5			○			
	T6			○	○		
	T7		○	○			
	T8		○	○	○		
#3 Pre-germinative test with KNO ₃ and IM	T1	○					
	T2					○	
	T3			○			
	T4			○		○	
#4 Pre-germinative test with GA	T1	○					
	T2						○

where G (%) is the percentage of germinated seeds, g_t is the number of germinated seeds 21 DAS, g_0 is the number of sown seeds, and v is viability observed in TTZ test.

To determine the dormancy period duration, the % G obtained was fitted to a non-linear function (Eq. 2):

$$\%G = G_i \cdot e^{Gt \cdot t} \quad (\text{Eq. 2})$$

where % G is the percentage of germinated seeds, G_i is the percentage of germinated seeds at the beginning, G_t is germination rate, and t is time as weeks after seed collection (WAC).

The goodness-of-fit measurements were based on the root-mean-square error (RMSE), where a lower value indicates a better model fit (Chantre et al., 2018). Percentage data were transformed by root arc-sin method and then ANOVA was run in InfoStat v2020 (Di Rienzo et al., 2020). Multiple comparisons were performed through the LSD test ($\alpha=0.05$).

RESULTS AND DISCUSSION

The TTZ test showed that *E. colona* seeds had a viability of 72 %. This value was maintained up to the end of the experimental period, which allowed using the same sample size in all the experiments.

Duration of dormancy period

Seed germination started 21 WAC in both samples, which indicates that seed dormancy persisted until that moment. The exponential model fitted (Eq. 2) showed that WW increased the final number of germinated seed by 25% and 59% in S1 and S2, respectively, compared with untreated seeds (Fig. 1).

The results indicate that the seed dormancy period of *E. colona* under storage conditions extended up 21 wk after seed collection. This does not agree with the results reported by Bazzigalupi and Picapietra (2015) who reported a 7-month dormancy period (approximately 30 wk). This variation can be attributed to a characteristic of the tested sample in each study, i.e., both harvest time and environmental conditions could affect seed size and germinative response (Soltani et al., 2018; De Long et al., 2019).

KNO₃, DE and WW treatments

The factorial model analyzed was significant ($p < 0.05$), with an acceptable fit in S1 ($r^2 = 0.66$) and S2 ($r^2 = 0.77$). Through the comparison of the three variables alone, only KNO₃ significantly improved the germination response ($p < 0.05$).

In addition, the effects of WW and DE were not significant as a single effect or in the interactions DE ' WW, KNO₃ ' WW, and KNO₃ ' DE.

For S1, no significant differences were found for the triple interaction KNO₃ ' DE ' WW ($p > 0.05$). Only KNO₃ significantly improved the germination response by 88%. On the other hand, KNO₃ in S2 showed a 126% improvement in germination response, while there were significant differences for the triple interaction ($p > 0.05$). KNO₃ alone (50.0%), KNO₃ plus WW (45.2%) and KNO₃ plus DE (57.1%) showed no significant differences in the percentage of *E. colona* germinated seeds. Furthermore, the effect of DE plus WW (33.3%) was not modified when KNO₃ was added (35.7%) (Fig. 2).

Several studies have indicated that the use of KNO₃ has an important effect on seed germination (Lambers and Oliveira, 2019; Pereira et al., 2020). However, nitrate availability is decisive for seed dormancy (Shu et al., 2016) and its positive effect on dormancy alleviation could be mediated by abscisic acid (ABA) metabolism. According to our results, *E. colona* seeds have a positive response to exogenous NO₃⁻. This could mean that this specie responds to the mechanism described by Matilla et al. (2015) where NO₃⁻ is a positive endogenous GA regulator.

The WW for 1.5 min was an effective treatment in promoting a greater number of *E. colona* germinated seeds, which agrees with Bazzigalupi and Picapietra (2015). After 33 wk of seed storage, WW was not an effective treatment to increase the number of germinated seeds. Although there was a positive effect on seed germination by WW in the first experiment, this was not observed after 33 wk. The optimal effect of WW is assumed to be between 21 and 33 WAC, which would be related to an acceleration in post-harvest maturation since no significant effect was observed after this period.

There is no restriction effect in germination due to the persistence of glumes in the fruit, meaning that DE is not a specific treatment for breaking seed dormancy of *E. colona*. It is well known that seed dormancy may be imposed by glumes in some species (Acar et al., 2017; Visser and Beaugendre, 2019), but this would not be the case in *E. colona*.

KNO₃ and water immersion (IM) treatments

The factorial model analyzed was significant ($p < 0.05$). The most important global effect on germination in both seed samples was given by the addition of KNO₃ (Fig. 3). Regarding S1, IM only had effects at 7 DAS, while KNO₃ significantly improved the germination response ($p < 0.05$) by 114% and 90% at 16 and 22 DAS,

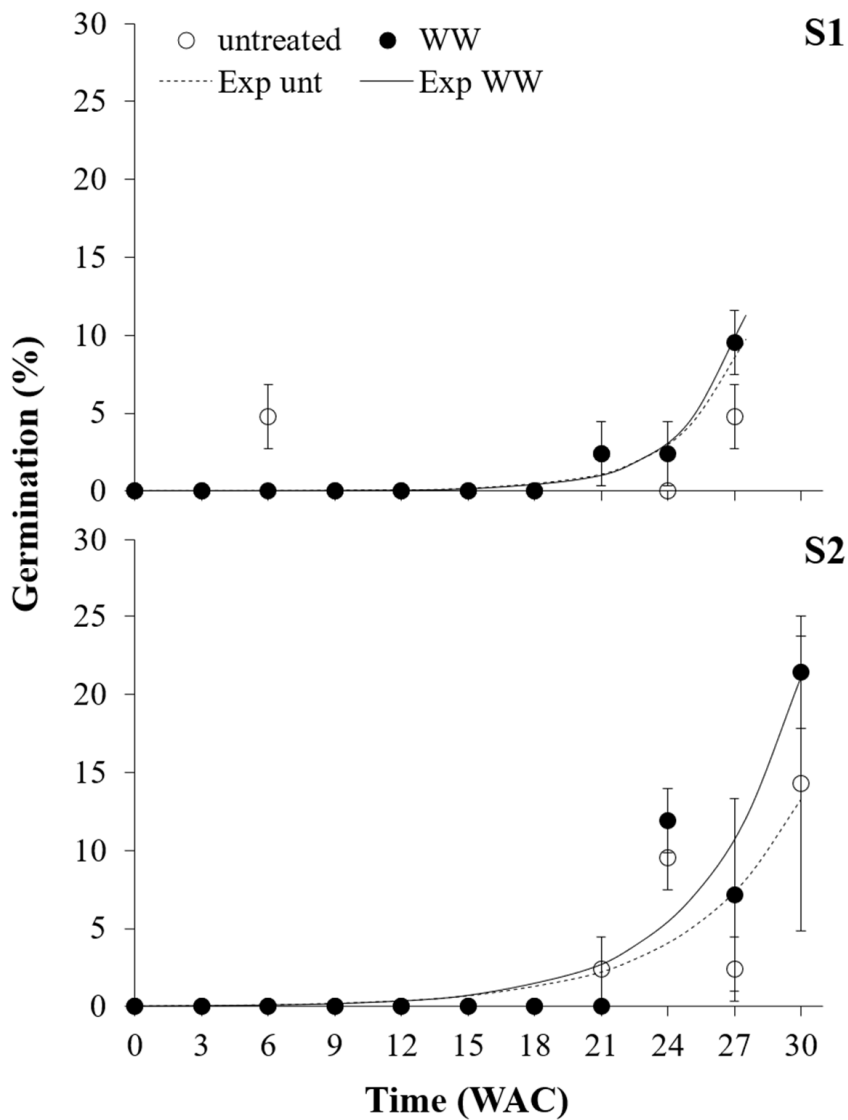


Fig. 1. Germination rate of *Echinochloa colona* seeds at 21 d after sown as a function of time expressed in weeks after seed collection (WAC). Average values of untreated seeds (open circles) and hot-water-washed seeds (WW, filled circles) with their respective standard deviations and the exponential model fit (Exp). Seed sample 1 (S1): untreated seeds (%G = $0.001 e^{(0.334 t)}$) and with water washing at 70 °C (%G = $0.001 e^{(0.366 t)}$). Seed sample 2 (S2): untreated seeds (%G = $0.036 e^{(0.197 t)}$) and with water washing at 70 °C (%G = $0.024 e^{(0.226 t)}$).

respectively. A similar effect by KNO_3 (91% and 105%, respectively) was observed in S2. The effect of IM on S2 germination percentage was significant at 7 DAS ($p < 0.05$), showing twice as many germinated seeds as those observed without this treatment. The IM of seeds showed an accelerated response at 7 DAS. This effect can be explained as germination inductions due to the previous imbibition, while imbibition is a necessary process to activate germination

mechanisms. However, IM is not effective for all species (Andrade and Laurentin, 2015). Even though this difference was observed at the beginning, the effect of IM was diluted at 22 DAS. This may also account for the fact that DE was not significant. In addition, glume permeability was not an obstacle to germination.

Gibberellic acid (GA) treatment

The use of GA as seed treatment to break

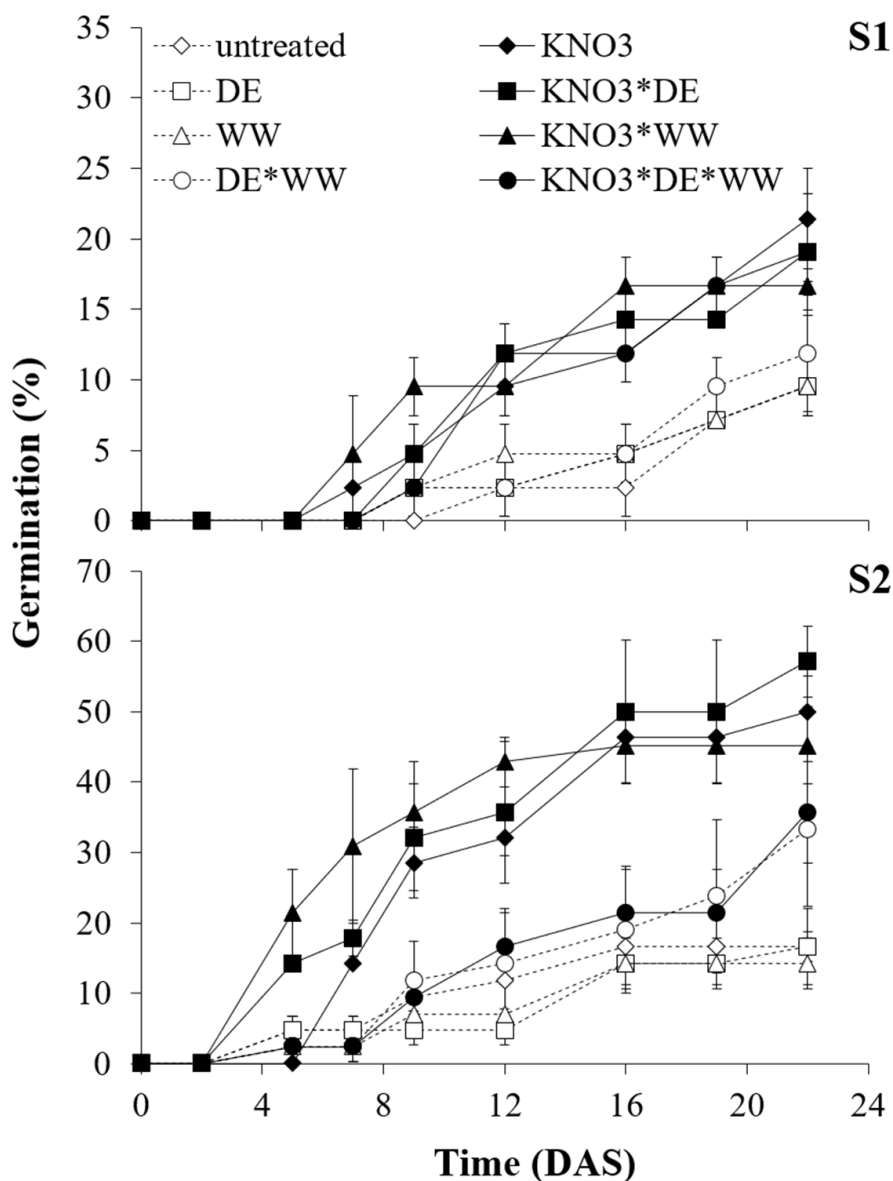


Fig. 2. Germination rate of *Echinochloa colona* seeds from a monoculture crop (S1) and a 3-yr rotation crop (S2) as a function of the time expressed in days after sowing (DAS) for the different treatments and interactions: negative control (untreated), dehulling (DE), hot-water washing (WW) and potassium nitrate (KNO_3), with respective standard deviation.

dormancy did not increase the number of germinated seeds. No differences were observed between treated and untreated seeds in S1 or S2 (Fig. 4). In terms of physiological dormancy, Lambers and Oliveira (2019) have described that an increase in the ratio of GA:ABA hormones has a direct impact on dormancy break. In this experiment, however, the addition of exogenous GA showed no effect.

The GA can release dormancy and promote germination on seeds with coat dormancy (Lambers and Oliveira, 2019). Since there were no significant effects with DE or exogenous GA, these observations presume that dormancy is not imposed by glumes. It is important to highlight that GA promotes germination in seeds with non-deep physiological dormancy, but not in deep-dormant seeds (Baskin and Baskin, 2014).

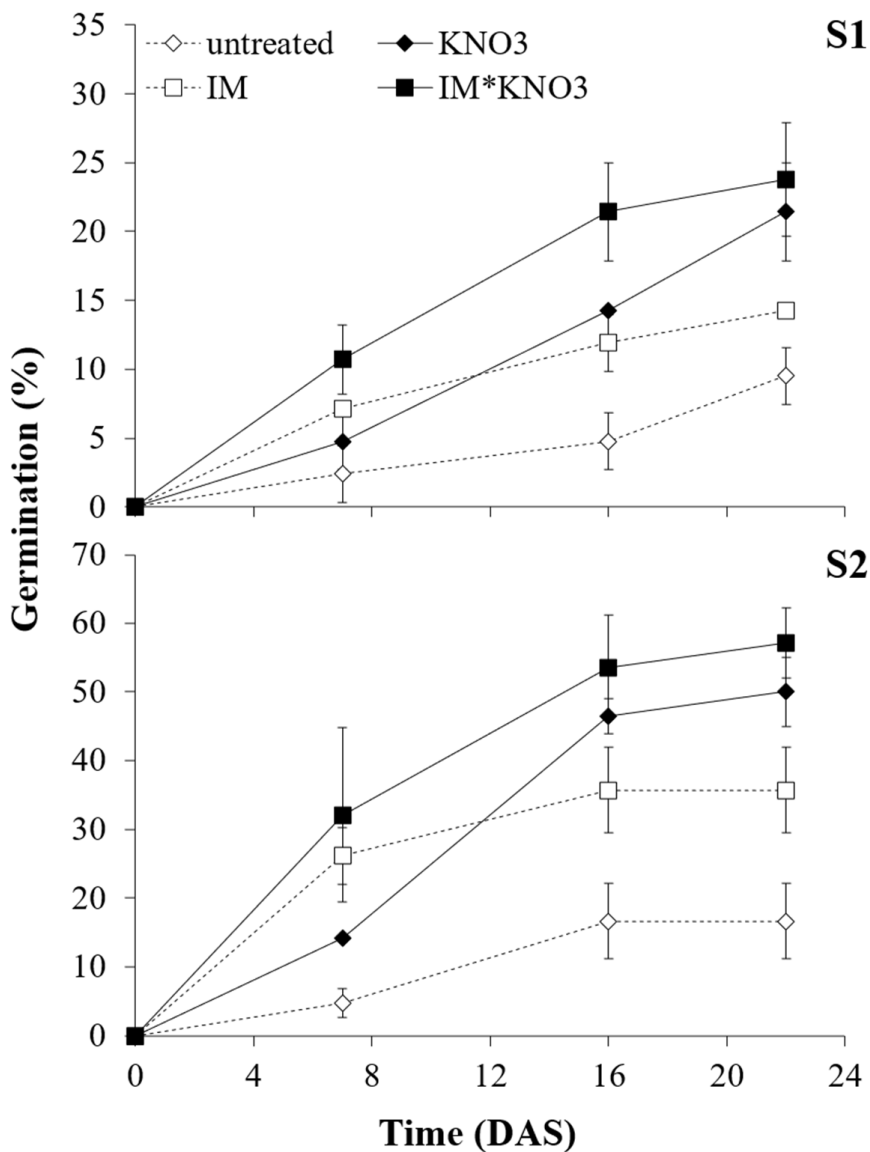


Fig. 3. Germination rate of *Echinochloa colona* seeds from a monoculture crop (S1) and a 3-year rotation crop (S2) as a function of the time expressed in days after sowing (DAS) for the different treatments and interactions: without treatment (untreated), potassium nitrate (KNO₃), immersion (IM), with respective standard deviation.

The results show that dormancy of *E. colona* seeds persists until 21 WAC. From this moment, the germination of seeds without treatment can gradually reach 7 – 19 %, while the addition of KNO₃ can result in a 2- or 3-fold increase in germination rate. This would indicate that the earlier seed collection occurs, the earlier germination. Likewise, a high germination rate could be achieved in soils with optimal N fertility.

CONCLUSIONS

As a treatment to overcome seed dormancy of *E. colona*, hot-water washing (WW) was ineffective immediately after seed collection. However, it showed a positive effect between 21 and 33 wk after collection, resulting in improved germinative response compared with untreated seeds. The addition of KNO₃ prior to sowing

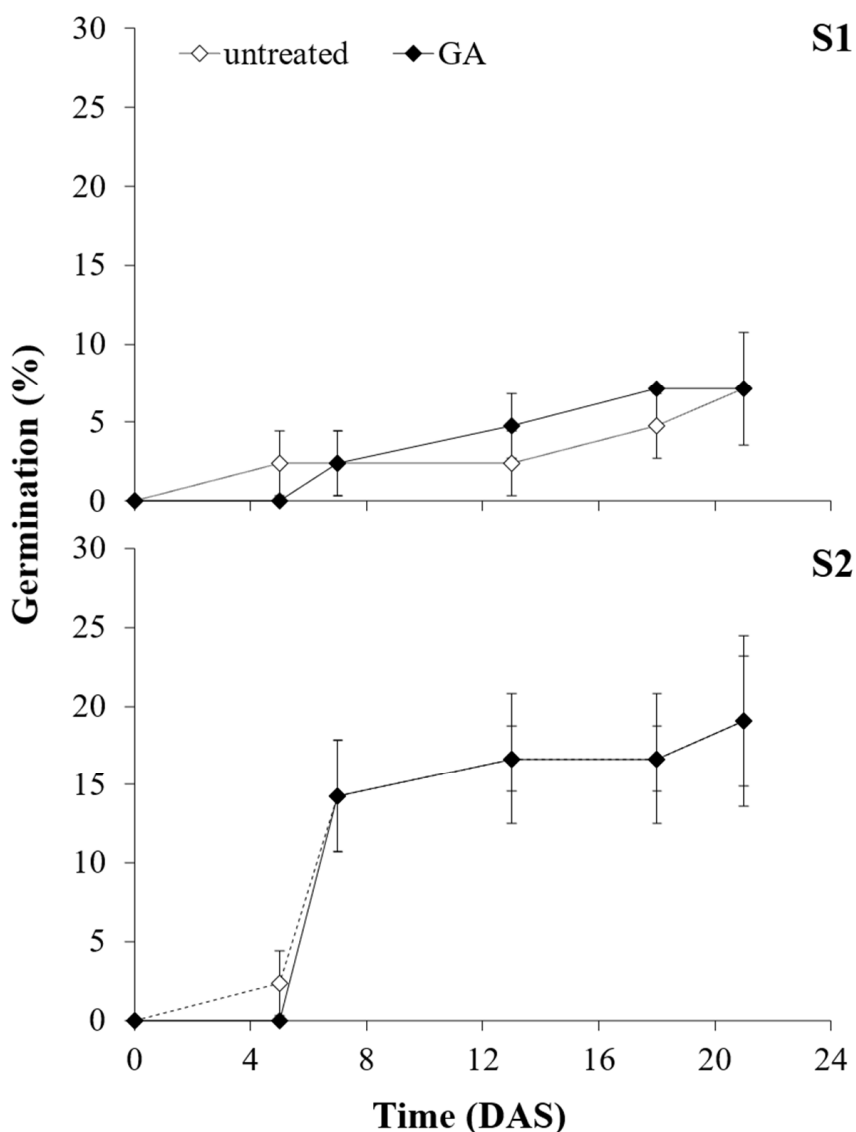


Fig. 4. Germination rate of *Echinochloa colona* seeds from a monoculture crop (S1) and a 3-yr rotation crop (S2) as a function of the time expressed in days after sowing (DAS) for untreated seeds and gibberellic acid (GA) treated, with respective standard deviation.

significantly increased the number of germinated seeds. Pre-germinative treatments WW, dehulling and exogenous gibberellic acid (GA) did not have a positive effect on the germinative response, while previous water immersion increased the number of germinated seeds immediately after sowing. The combined treatments under evaluation did not outperform the effect of KNO_3 alone. The obtained results allow concluding that *Echinochloa colona* seed dormancy is not related to glumes, as a physical barrier, and that KNO_3 could have a positive effect on GA:abscisic

acid regulation. Future research is required to determine the effect of exogenous GA and its combination with KNO_3 on seed germination.

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