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Research Note

Germination of *Bromus auleticus* after different treatments to release seed dormancy

Silvana Gonzalez[1] and Federico Condón[2]*

Seed and Plant Genetic Resources Unit, National Institute for Agricultural Research (INIA Uruguay), La Estanzuela Research Station, C.P 70000 Colonia, Uruguay
*Author for correspondence (E-mail: [1] sngonzalez@inia.org.uy; [2] fcondon@inia.org.uy)

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Abstract

Bromus auleticus is a cool season perennial C3 grass, recognised as a forage plant genetic resource and used for native grasslands restoration. It is native to the campos biome, found in southern Brazil, Uruguay and central Argentina. Its forage yield is comparable with tall fescue. Seed dormancy is a problem to evaluate germination and for commercialisation of this species. Using four recently harvested seed lots of three different genotypes, we tested six different treatments to release dormancy: a control (mean germination 52%); 0.05 and 0.1% gibberellic acid; KNO_3; pre-chilling + KNO_3; and pre-chilling (mean germination across seed lots and treatments, 87%). Pre-chilling + KNO_3 and pre-chilling were the best treatments to break dormancy, with mean germination times (MGT) reduced to half (8.7 and 9.3 days^{-1}) that of the untreated control (19.2 days^{-1}). The treatment with KNO_3 alone did not yield uniform results across seed lots; when combined with pre-chilling, final germination did not increase but showed more consistent results. The use of 0.05% gibberellic acid was less efficient than pre-chilling to reduce the MGT of 17.2 days^{-1}, but it could be considered as an alternative treatment for seed lots in which the germination results are needed fast and has the additional advantages of avoiding exposing seeds to cold stress. Furthermore, if seeds are contaminated with fungi, it reduces growth time and contamination effects.

Keywords: *Bromus auleticus*, dormancy release, germination, gibberellic acid, grass, KNO_3, seeds

Experimental and discussion

Bromus auleticus Trinius (ex Nees) or bromegrass is a cool-season, perennial, allogamous C3 grass, with a chromosome number of 2n = 6x = 42 (Martinello and Schifino-Wittmann, 2003; Artico *et al*., 2017), native to South America with natural distribution in Southern Brazil, Uruguay and central Argentina (Williams *et al*., 2011). It is recognised as a forage plant genetic resource (Rosengurt, 1946; Millot, 2001) with characteristics that include

productivity, similar to tall fescue during the second and third year after planting (Castro and Cuitiño, 2015a, b), tolerance to stresses, and persistence under controlled grazing management (Millot, 1969). It is considered a valuable species for grassland restoration in agricultural fields and in over-grazed natural pastures (Franco, 2019). Characterisation studies of morphological, reproductive and productive traits in Argentina (Gutierrez *et al*., 2006), Brazil (Scheffer-Basso *et al*., 2009) and Uruguay (Condón *et al*., 2017), have reported a significant variation in the species. Two cultivars ('Potrillo' and 'INIA Taboba') have been released (Bemhaja, 2001; Rivas, 2001) by the College of Agronomy and the National Agriculture Research Institute (INIA) of Uruguay.

Bromegrass germination has been observed in temperatures that range from 10 to 25°C, but they do not show consistent and rapid germination. The use of pre-chilling at 7°C for seven days (Ruiz *et al*., 2006; Gutierrez *et al.*, 2015) and recently H_2O_2 have been reported to increase germinability (Kindiger, 2019). The aim of this study was to test the use of gibberellic acid (GA_3) and KNO_3, two dormancy release treatments recommended by the International Seed Testing Association for various species (ISTA, 2017).

Seeds were harvested in November 2018 from two plots of the cultivar 'INIA Tabobá', identified as Taboba1 and Taboba2, respectively, and random samples were taken from the harvested lots for this study; seeds from two genebank accessions cultivated at the experimental field of INIA La Estanzuela, Colonia, Uruguay, for one generation, identified as URY11241 (collected from Colonia, Uruguay) and URY19976 (collected from Salto, Uruguay). After harvest, the seeds were kept at room temperature, at approximately 20°C and 70% relative humidity (RH) for two months, then they were threshed and treated with carbendazim (200 g l^{-1}) + thiram (200 g l^{-1}) + iprodione (100 g l^{-1}) at a dose of 200cc/ 100 kg of seeds for the control of fungi that may affect germination.

The treatments applied to the seeds were:

1) Control = distilled water;
2) GA005 = 0.05% solution of GA_3;
3) GA010 = 0.1% solution of GA_3;
4) KNO_3 = 0.2% solution of KNO_3;
5) Pre-chill + KNO_3 = 0.2% KNO_3 and 10 days of pre-chilling at 5 ± 2°C;
6) Pre-chill = distilled water and 10 days of pre-chilling at 5 ± 2°C.

For each treatment, four sub-samples of 50 seeds were germinated in Gerbox® boxes (115 × 110 × 35 mm) on top of two sheets of paper (Anchor Paper Co., 76 lb, 254 × 508 mm) imbibed with 8 ml of the solution corresponding to each treatment. Then, the seeds were put in a germination chamber at 20/30 ± 2°C, 90% RH and an 8/16-hour photoperiod (light/dark) for 28 days (ISTA, 2017). Germinated seeds were counted at 7, 14, 21 and 28 days after the trial initiation.

Germination indices and the statistical analysis were performed using the GerminaR package (Lozano-Isla *et al.*, 2019) in the R environment. The analysis applied was factorial ANOVA, with a Tukey test with alpha = 0.05 to determine if means were statistically different. The germination indices calculated were germination percentage (GRP) and mean germination time (MGT) (Labouriau, 1983), as a measure of germination speed, being equivalent to a half of the time needed to achieve final germination.

There were significant ($P < 0.01$) effects on germination of seed lot, dormancy treatment and the interaction of seed lot and dormancy treatment (figure 1A). The lots had different levels of dormancy: Taboba2 showed greater dormancy than Taboba1 and URY19976, that had similar dormancy levels. URY11241 showed a lower level of dormancy. The GRP increased significantly ($P < 0.05$) from 52% for the control to 87% across all dormancy-breaking treatments for all the seed lots except for URY11241, which in all cases achieved similar levels of final germination (not statistically different), explaining the seed lot by treatment interaction.

The pre-chill + KNO_3 treatment resulted in the most consistently fast germination (mean MGT 8.7 day^{-1}), although not significantly different from the pre-chill (average 9.7 day^{-1}) treatment (figure 1B), a result consistent with previous reports (Ruiz *et al.*, 2006). The KNO_3 treatment had a MGT average of 17.1 day^{-1} and GA005 and GA010 (mean MGT 14.7 and 15.5 day^{-1}, respectively). The URY11241 seeds reached a GRP higher than 70% at seven days of incubation time, the other seed lots reached 50% germination at 10 days, while for the control, this germination value was achieved after 20 days of incubation.

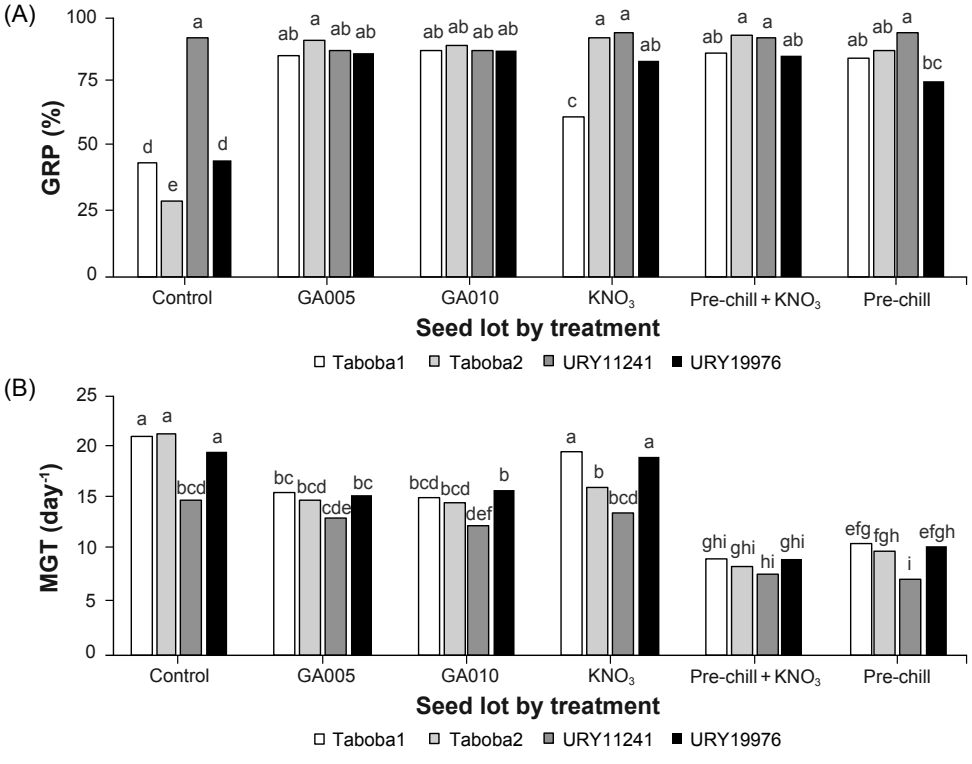

Figure 1. (**A**) Final germination (GRP) and (**B**) mean germination time (MGT) for four seed lots of *Bromus auleticus* in response to different dormancy-breaking treatments. Bars with the same letter are not different at $P < 0.05$ according to the Tukey test. Treatments: Control = distilled water; GA005 = 0.05% GA_3; GA010 = 0.1% GA_3; KNO_3 = 0.2% KNO_3; Pre-chill KNO_3 = 0.2% KNO_3 and 10 days of pre-chilling at 5 ± 2°C; Pre-chill = distilled water and 10 days of pre-chilling at 5 ± 2°C.

The effect of KNO_3 on germination and on MGT depended on the seed lot. For Taboba1, the increase in germination was significantly lower ($P < 0.05$) than the increase observed for the other lots and the MGT was not reduced except for Taboba2 seeds (figure 1). Although seed analysis laboratories in Uruguay use the combination of KNO_3 with pre-chill as a treatment to enhance dormancy breakdown, we did not observe a significant response in GRP or MGT when comparing the pre-chill and the KNO_3 treatments.

The GA_3 treatment promoted germination, although it was less efficient than pre-chilling in the reduction of the MGT. At 14 days, average GRP was 74% for 0.05% GA_3 and 83% for pre-chilling, suggesting that the dormancy of this species could be hormonal in nature (Ruiz *et al*., 2006). The GA_3 concentration increase from 0.05 to 0.1% did not change the response. The use of GA_3 could be considered as a treatment when the germination and the vigour are low, reducing the risk of damage due to imbibition at low temperatures (Olivares *et al*., 1990), or if more rapid germination is required due to the presence of fungi. The greater efficiency of GA_3 in the breakdown of dormancy compared with KNO_3 is due to the fact that the former is directly involved in the control and promotion of germination (Gashi *et al*., 2012).

We can conclude that overcoming dormancy in *Bromus auleticus* seeds is a time-consuming process. Pre-chilling is the most efficient method to reduce the MGT but increasing the pre-treatment time in five days; alternative methods like the use of GA_3 instead of pre-chilling can reduce the MGT without increasing the total time for the analysis.

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