# EFFECT OF EXOGENOUS ABA AND FLURIDONE ON THE GERMINABILITY OF *BUGLOSSOIDES ARVENSIS* L SEED PROGENY UNDER CONTRASTING MATERNAL NITROGEN LEVELS

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## SUMMARY

Abscisic acid (ABA) is one of main physiological dormancy regulators. During embryogenesis a hormone peak determines dormancy level. Thereafter, ABA concentration and seed embryo sensitivity have a crucial role blocking germination. Unfavorable germination conditions might promote *de novo* ABA production impeding germination. Maternal effects might generate differences in progeny dormancy. *Buglossoides arvensis* is a facultative annual weed with differences in physiological dormancy level due to maternal nitrogen fertilizer. The aim of the present work was to evaluate the effect of exogenous ABA and its inhibitor (fluridone, FLU) on the germinability of *B. arvensis*. A $F_2$ progeny (from fertilized and without fertilizer plants: cN and sN, respectively) was tested under ABAand FLU gradients at 7, 15 and 20 °C during 30 days. Dose-response curves were fitted and curve parameters compared by ANOVA. Dose-response curves showed that both accessions (sN and cN) expressed a similar seed germinability. At supra-optimal temperatures FLU stimulated germination. More studies would be necessary to clarify the maternal fertilization effect on germination, its hormonal regulation and its consequences on weed emergence cohorts in the field.

**Keywords:** *Lithospermum arvense* L., Corn Gromwell, transgenerational effects, hormones, nitrogen.

## RESUMEN

El ácido abscísico (ABA) es uno de los principales reguladores fisiológicos de la dormición seminal. Durante la embriogénesis un pico de ABA determina el nivel de dormición. Desde ese momento, la concentración de dicha hormona y la sensibilidad del embrión hacia ella adquieren un rol clave en el bloqueo de la germinación. Condiciones desfavorables para la germinación pueden estimular la producción *de novo* de ABA en la semilla impidiendo el desarrollo de dicho proceso. A su vez, diferencias en el nivel de dormición pueden ser causados por efectos maternos. *Buglossoides arvensis* es una maleza anual facultativa con diferencias en el nivel de dormición fisiológica debido a la fertilización nitrogenada a la que la planta madre fue expuesta. El objetivo del presente trabajo se basó en evaluar el efecto del ABA exógeno y su inhibidor (fluridona, FLU) sobre la germinabilidad de *B. arvensis*. Se evaluó una progenie $F_2$, proveniente de plantas madres con y sin fertilización nitrogenada (cN y sN, respectivamente), bajo gradientes de ABA y FLU a 7, 15 y 20 °C durante 30 días. Se ajustaron curvas de dosis-respuesta y sus parámetros fueron comparados mediante ANOVA. Ambas accesiones (cN y sN) expresaron patrones de germinación similares. Bajo temperatura óptima, la germinación se encontró estimulada por FLU. Serían necesarios más estudios para aclarar el efecto de la fertilización materna sobre la germinación, su regulación hormonal y sus consecuencias sobre las cohortes de emergencia de malezas en el campo.

**Palabras clave:** *Lithospermum arvense* L., Corn Gromwell, efectos transgeneracionales, hormonas, nitrógeno.

## INTRODUCTION

The balance between abscisic acid (ABA) and active gibberellins (GAs) plays a key role in physiological dormancy, being the endogenous ABA peak, which occurs during embryogenesis, the determinant of post-harvest dormancy level[1]. The amount of ABA present during imbibition is the main conditioning for germination which can be impeded if the hormone reaches a certain inhibitory threshold[2], a common situation in dormant seeds. It was described an increment in the level of endogenous ABA in dormant seeds of *Nicotiana plumaginifolia* after imbibition which did not occur in after-ripened seeds[3].

Fluridone (FLU) (1-methyl-3-phenyl-5- [3-trifluoromethy1 (phenyl)] - 4- (l //) - pyridinone) is an inhibitor in the biosynthesis of carotenoids, the main precursors of ABA in plants[4]. Due to ABA *de novo* synthesis inhibition, fluridone stimulates germination.

Hormones levels and their sensitivity are influenced by the prevailing environmental conditions during both embryogenesis and seed imbibition. It was described the germinative differences in *Sorghum bicolor* embryos from mothers subjected to water stress versus control conditions were linked to the ABA level[5].Unfavorable environmental conditions for germination may be associated, although partially, with an increment of embryo sensitivity and/or ABA concentration.At high temperatures, non-dormant seeds of *Lactuca sativa* increased their sensitivity to ABA 6-9-fold and the hormonal content was much higher than in the optimal germination temperature[6].However, when the imbibition solution contained FLU, the germination reached almost the maximum percentages decreasing the amount of registered ABA. Therefore, in *L. sativa* a continuous synthesis of ABA would be necessary to maintain the inhibition of germination at high temperatures[7].

*Buglossoides arvensis* L. is a facultative winter annual weed with non-deep physiological dormancy. Its germination is affected by maternal nitrogen fertilization[8]. The aim of the present work was to evaluate the effect of exogenous ABA and its inhibitor (fluridone, FLU) on the germinability of *B. arvensis* seed accessions generated under contrasting maternal nitrogen levels. A $F_2$ progeny was tested under ABAand FLU gradients at 7, 15 and 20 °C during 30 days.

## METHODS AND MATERIALS

### Seed production

*Buglossoides arvensis* mature seeds ($F_1$) were hand-collected from two *Avena sativa* field plots, an unfertilized (0 Kg $N^{-ha}$) and a nitrogen fertilized plot with UREA (100 Kg $N^{-ha}$), located at the Experimental Station of INTA-Bordenave (37°50055″S, 63°01020″W), Buenos Aires, Argentina (December 2013). Seeds were dry stored until August 2014, when the field trial was established at the experimental site of CERZOS-CONICET (38°39´54"S, 62°13´58"W) in Bahia Blanca.

$F_1$ seeds were seeded at the experimental site of CERZOS-CONICET following a completely randomized factorial design with four replicates. The experimental design consisted on two levels of nitrogen fertilization (0 Kg N $ha^{-1}$: sN; 150 Kg N $ha^{-1}$: cN) for plants obtained from the $F_1$. Nitrogen fertilization was performed with UREA (46% N) splitted between the vegetative growth stage and the flowering state. $F_2$ mature seeds were harvest in January 2015 and stored under laboratory conditions (22 ± 2 °C) until the initiation of the germination tests three weeks after harvest.

### Germination test

$F_2$ seeds obtained under both unfertilized and fertilized conditions were incubated at sub- (7 °C), optimal (15 °C) and supra-optimal (20 °C) temperature. Thirty seeds of each accession (sN or cN) were placed on two filter paper sheets in 55-mm diameter plastic Petri dishes and moistened with 4 ml of solution. The drugs used were ABA (ABSCISIC ACID PLANT CELL 98.5% HPLC SIGMA) and fluridone (FLURIDON PESTANAL, FLUKA). They were diluted in distilled water with solvent (50 mg ABA/ml methanol or 1% acetona - $H_2O$ $_v/^v$ respectively) until reaching the concentrations of 0.01; 0.1; 1; 10; 100 and 1000 µM[6]. A preliminary trial revealed that the doses used of each solvent did not interfere with germination under any of the conditions tested. A completely randomized factorial design with three replicates was applied.

Seeds were scored for germination every two days during 30 days. At the end of the experiments, a crush-test[9] was carried out to determine the viability of the remaining seeds. The final germination percentage was calculated on the basis of the total number of viable seeds.

### Statistical analysis

Germination figures were analyzed with one-way ANOVA and *post hoc* Tukey´s test was conducted. Arcsine transformation was utilized for homoscedasticity. $F_2$ seed accessions were described by nonlinear regression analysis of dose-response curves. The $I_{50}$ was calculated (dose that causes an increase or decrease of 50% with respect to the control).

A preliminary analysis revealed that Weilbull model with the lower limit equal to zero, expresses the best fit in all cases [eq. 1]:

$$Y = d^{\#} \exp\{-\exp[b(\log x - e)]\} \qquad \text{[eq. 1]}$$

Where *c* is the lower and *d* the upper limit. The parameter *b* denotes the relative slope around *e*, the inflexion point.

The program R with the *drc* extension package was used. Simultaneously multiple dose-response curves were fitted for both $F_2$ accessions incubated at each medium and temperature. Curve parameters were compared by ANOVA for significant differences.

## RESULTS AND DISCUSION

$F_2$ fertilized accessions showed higher germination percentages at suboptimal temperature different ($p$=0.0051). At 15 and 20 ºC germination also showed higher figures at cN but were not statistically significant.

Seed imbibition with ABA showed to be effective reducing the germination until inhibiting it in all the temperatures tested (Figure 1). At sub- and optimal temperature both accessions expressed a similar behavior but cN was more sensitive specially under low doses. At 20 °C germination was very low throughout the range of concentrations without being able to adjust a dose-response curve. The cN behaviour suggests that a higher GAs sensitivity and/or production would be necessary to counteract their effect. This result generated the hypothesis that cN seeds could have a differential affinity of receptors towards the ABA molecule[10], since varying nitrogen availability could influence the composition of the proteins present in the membranes. Other aspects that could be influenced by the maternal environment include receptor capacity of occupation and the intake efficiency and transport to the site of action[10].

Seeds incubation on FLU showed similar effects in both accessions (Figure 1) stimulating the germination in all the contexts. This fact is an indication that there is *de novo* ABA production during imbibition. At the average temperature germination was abruptly reduced to 1000 µM, hence these points were excluded to analysis. This fact could be due to some phytotoxic effect from the herbicide. At 20 °C the germination doubled with respect to the control. The fact that $G_{max}$ is around 40% suggests that the thermo-inhibition observed in this species is not fully due to the *de novo*

ABA production. The fact that the application of fluridone does not achieve a stimulation of 100%, especially in sN, may be due to the presence of some embryonal ABA. In this way, even though the production *in situ* is completely inhibited, from having high levels of ABA since the embryogenesis, the seeds will not germinate

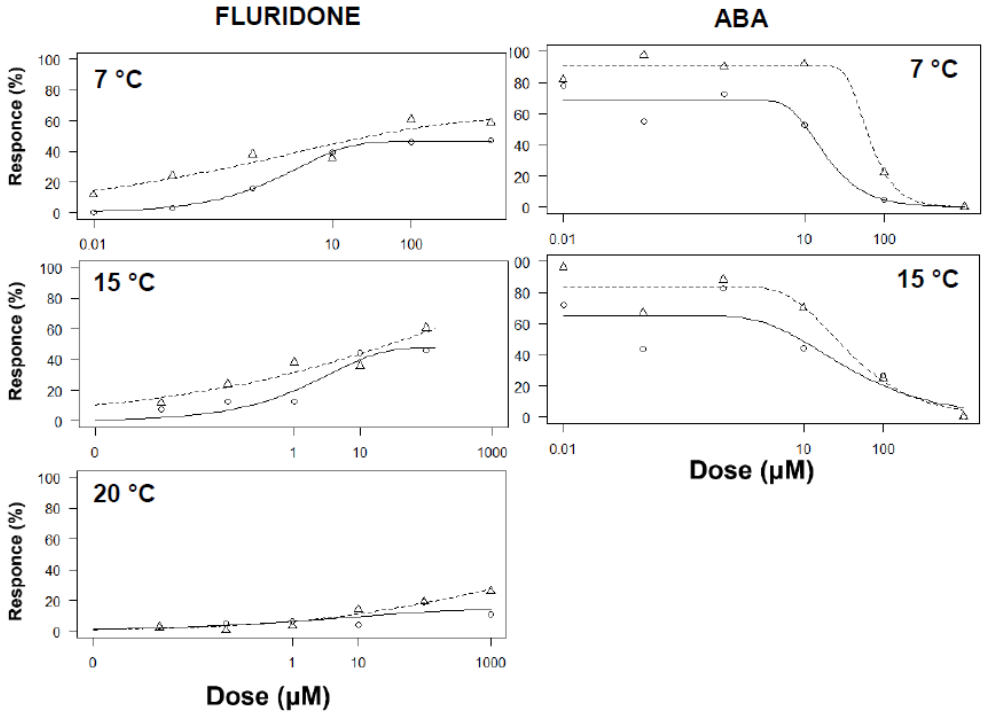


**Figure 1** Log dose-response curves for fertilized (---) and non-fertilized ( ) seeds imbibed with Abscisic acid (ABA) and fluridone under 7, 15 and 20 °C.

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**Table 1.**Dose-response curve parameters (*c*: lower limit, *d*: upper limit, *d*: slope, *e*: inflection point) and *p*-values between accessions for Abscisic acid (ABA) and fluridon (FLU) under sub- (7 °C), supra- (20 °C) and optimal (15 °C) temperature. $I_{50}$ stands for the effective dose that produces a response of the 50%.

| T° | Drug | Model fit ($R^2$) | *c* | *d* | *b* | e | $I_{50}$ |
|---|---|---|---|---|---|---|---|
| **7 °C** | ABA | 0.8748 | c=0 | *p*=0.0164* $d_{sN}$=68.49 $d_{cN}$=90.45 | *p*=0.9365$^{ns}$ c=0.6417 | *p*=0.4996$^{ns}$ e=0.2512 | *p*=0.8199$^{ns}$ $I_{50}$=3.6013 |
| **15 °C** | ABA | 0.082 | c=0 | *p*=0.0402* $d_{sN}$=65.42 $d_{cN}$=83.69 | *p*=0.522$^{ns}$ c=0.7459 | *p*=0.7398$^{ns}$ e=0.7625 | *P*=0.9150$^{ns}$ $I_{50}$=1.1146 |
| **7 °C** | FLU | 0.8972 | c=0 | *p*=0.2825$^{ns}$ d=0.6696 | *p*=0.5093$^{ns}$ b=3.336 | *p*=94.78 e=1.3555 | *p*=0.9292$^{ns}$ $I_{50}$=0.7377 |
| **15 °C** | FLU | 0.771 | c=0 | *p*=0.4184$^{ns}$ d=0.4652 | *p*=0.5532$^{ns}$ b=2.9598 | $e_{sN}$=3.3696 $e_{cN}$=285.44 *p*<0.0001**** | *p*=0.9331$^{ns}$ $I_{50}$=21.6439 |
| **20 °C** | FLU | 0.7598 | c=0 | $d_{sN}$=3.1934 $d_{cN}$=14.777 *p*=0.01798* | *p*=0.9962$^{ns}$ b=0.9947 | $e_{sN}$=0.0251 $e_{cN}$=0.9713 *p*<0.0001**** | *p*=0.8287$^{ns}$ $I_{50}$=96.3940 |

## CONCLUSIONS

It might be speculated that Nitrogen supply to the mother plants could affect membranes´ proteins composition influencing hormone sensitivity and in this way dormancy level. In the other hand, fluridone would not be entirely effective to terminate dormancy in *Buglossoides arvensis* seeds. In this way, the thermo-inhibition is not only caused by *de novo* ABA synthesis. Further studies should be performed to test this hypothesis.


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