EFFECT OF GA ON THE GERMINABILITY OF *BUGLOSSOIDES ARVENSIS* L SEED PROGENY UNDER CONTRASTING MATERNAL NITROGEN LEVELS

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SUMMARY

Maternal nitrogen level during seed formation influence seed dormancy and germination of the seed progeny. *Buglossoides arvensis* L. shows non-deep physiological dormancy regulated by ABA-GA balance. The aim of the present work was to evaluate the effect of exogenous GA$_3$ and its inhibitor (Paclobutrazo, PAC) on the germinability of *B. arvensis*. F_2 seed progeny obtained under contrasting maternal nitrogen levels (sN, 0 kg N ha^{-1} and cN, 150 kg N ha^{-1}) was tested under GA$_3$ and PAC gradients at 7, 15 and 20 °C during 30 days. Dose-response curves were fitted and curve parameters compared by ANOVA. F_2-cN showed higher germination percentages at different incubation temperatures. For both accessions the germination percentages increased with GA$_3$ concentration. However, no statistical differences among Sn and Cn were observed. For both accessions, germination was statistical reduced by PAC. At 7 °C cN expressed an eight-fold higher I_{50} value (I_{50cN}= 52.15µM, I_{50sN}= 6.33 µM). These results suggest that observed differences in final germination were not due to differences in GA$_3$ sensitivity among accessions. The dose-response curves would indicate a tendency for sN to be more sensitive to PAC at sub-optimal temperature. 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 nivel de nitrógeno disponible por la planta madre durante la formación de la semilla influye en el nivel de dormición. *Buglossoides arvensis* L. expresa dormición fisiológica no profunda regulada por el equilibrio ABA-GA. El objetivo del presente trabajo fue evaluar el efecto de GA$_3$ exógeno y su inhibidor (Paclobutrazo, PAC) sobre la germinabilidad de *B. arvensis*. Una progenie F_2 obtenida bajo niveles de nitrógeno materno contrastantes (sN, 0 kg N ha^{-1} y cN, 150 kg N ha^{-1}) fue embebida bajo gradientes GA$_3$ y PAC a 7, 15 y 20 °C durante 30 días. Se ajustaron curvas de dosis-respuesta y sus parámetros fueron comparados mediante ANOVA. F_2-cN expresó mayores porcentajes de germinación a diferentes temperaturas de incubación. Para ambas accesiones, los porcentajes de germinación aumentaron con la concentración de GA$_3$. Sin embargo, no se observaron diferencias estadísticas entre Sn y Cn. Para ambas accesiones, la germinación fue estadísticamente reducida por PAC. A 7 °C, cN expresó un valor de I_{50}ocho veces mayor (I_{50cN} = 52.15 µM, I_{50sN}= 6.33 µM). Estos resultados sugieren que las diferencias observadas en la germinación final no se debieron a diferencias en la sensibilidad de GA$_3$ entre las accesiones. Las curvas de dosis-respuesta indicarían una tendencia a una mayor sensibilidad al PAC por parte de sN bajo temperatura subóptima. 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

Seed dormancy and germination are regulated by hormones, primarily abscisic acid (ABA) and active gibberellins (GAs) which overall balance determines seed germinability[1]. Seed germinability depends on both the concentration on seed tissue and the embryo sensitivity to such hormones which vary according to environmental or genetic conditions[2]. ABA is involved in the induction of primary dormancy during seed maturation[3] and the maintenance of the dormant state on imbibed seeds after shedding[4]. GAs are important for the promotion of germination once ABA-mediated inhibition was overcome[5]. To allow germination GAs causes an increment on the embryo growth potential and a reduction on the resistance of the endospermic enclosing structures[5]. The action of GAs requires a receptor whose sensitivity depends on temperature, consequently, germination is restricted to those periods that the receptor is available or active[6].

It is widely accepted that temperature regulates both dormancy and germination[1]. In facultative annual species a mayor emergence fraction of the population occurs during autumn while a second cohort is restricted to early spring previous to soil temperature increment which impedes subsequent field emergence. In fact, facultative winter annual seeds could be influence by thermo-inhibition germinate due to high temperature[7].

Buglossoides arvensis L. (corn gromwell) is a winter facultative annual weed of South-west Buenos Aires province. In previous studies, differences were noticed in *B. arvensis* dormancy because of the level of maternal nitrogen fertilizer[8]. The objective of the present work was to evaluate the effect of exogenous GA$_3$ and its inhibitor (Paclobutrazol) on the F_2 germinability of *B.*

arvensis seed progeny obtained under contrasting maternal nitrogen levels (sN, 0 kg N ha^{-1} and cN, 150 kg N ha^{-1}).

MATERIALS AND METHODS

Seed production

Buglossoides arvensis mature seeds (F_1) were hand-collected from two *Avena sativa* field plots, an unfertilized (0 Kg N ha^{-1}) and a nitrogen fertilized plot with UREA (100 Kg N ha^{-1}), 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 (sN and cN) were incubated at sub- (7 °C), optimal (15 °C) and supra-optimal (20 °C) temperature with PAC or GA_3 (0.01, 0.1, 1, 10, 100 y 1000 μM[9]. Three replicates of 30 seeds each were sown on two filter paper in 5.5 cm diameter plastic Petri dishes. Gibberellic acid or PAC were dissolved in ethanol (50 mg GA_3/ml ethanol) or acetone (0.1 %), respectively.

Seeds were scored for germination every two days. At the end of the experiments, a crush-test[10] 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. A preliminary analysis revealed that Weibull model expresses the best fit in all cases [eq. 1], except for GA_3 at 7 °C where the lower limit equals zero [eq. 2]:

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

$$Y = d^{\#} \exp\{-\exp[b(\log x - e)]\} \qquad \text{[eq. 2]}$$

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 different incubation temperatures (Figure 1), but differences among sN and cN were observed only in the sub-optimal germination condition (p=0.0051).

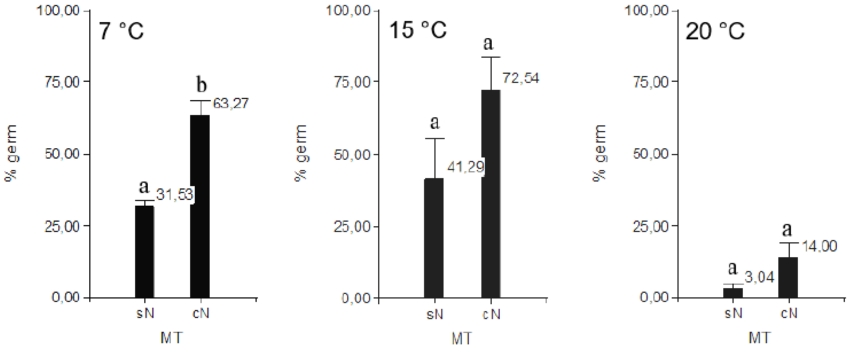

Figure 1 Re-transformed final germination percentages (±SE) for F_2 seeds obtained under two maternal nitrogen fertilization treatments (MT): sN, 0 kg N ha^{-1} and cN, 150 kg N ha^{-1}. Means designated with the same letter are not significantly different (p<0.05) according to Tukey´s test.

For both accessions and under the three incubation temperatures, the germination percentages increased as the GA_3 concentration increased, however, without statistical differences between Sn and Cn accessions, p>0.05, (Table 1). Nevertheless, at optimal temperature differences were observed in the mean lethal dose (p<0.001) expressing sN a higher value (Figure 2; Table 1). These results suggest that observed differences in final germination were not due to differences in

GA_3 sensitivity among accessions. However, at 20 °C it appears to be a higher GA_3 response in the fertilized F_2 accession which might increase the amount of emerged seedlings during early spring.
For both accessions, germination was statistical reduced by PAC (Table 1; Figure 2). At supra optimal temperature, no germination was observed (data not shown). At 7 °C the fertilized subpopulation expressed an eight-fold higher I_{50} value (I_{50cN}= 52.15μM, I_{50sN}=6.33μM) although such differences were not statistically significant (Table 1). The dose-response curves would indicate a tendency for sN to be more sensitive to PAC at sub-optimal temperature. The germinative difference between both accessions could be linked to a greater amount requirement of GA_3. This result coincides with that observed in *Arabidopsis* where seeds derived from plants with higher nitrogen fertilization showed to be slightly more insensitive to paclobutrazol[11]

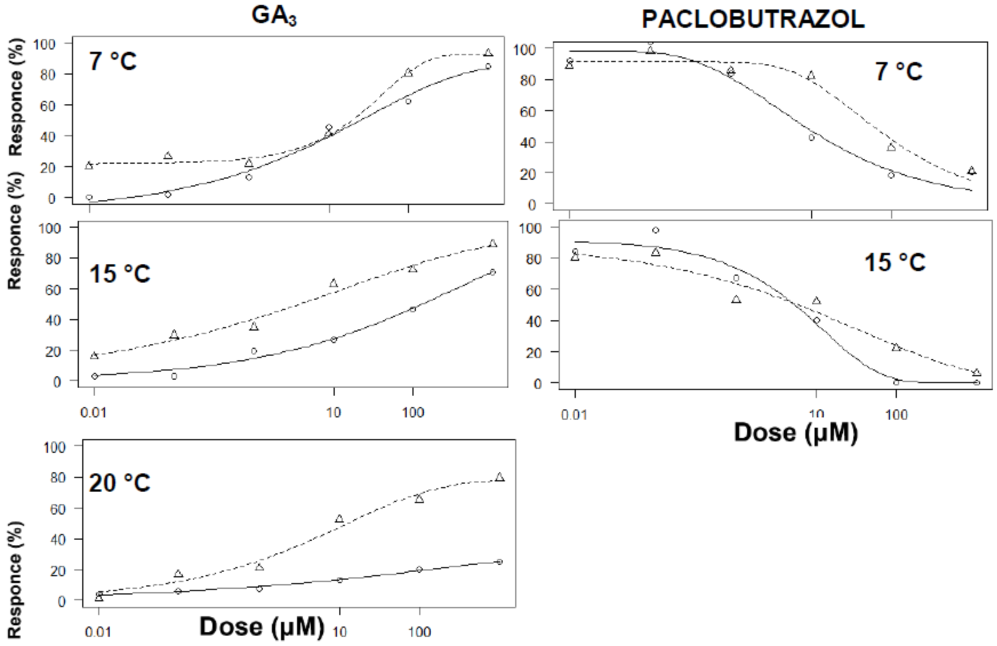

Figure 2 Logdose-response curves for fertilized (---) and non-fertilized (—○—seeds imbibed with Gibberellic acid (GA_3) and Paclobutrazol under 7, 15 and 20 °C.

Table 1. Dose-response curve parameters for F_2 accessions (*c*: lower limit, *d*: upper limit, *d*: slope, *e*: inflection point) and *p*-values for gibberellic acid (GA_3) and paclobutrazol (PAC) 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	AG_3	0.9318	p=0.1254^{ns} c=-0.4309	p=0.8488^{ns} d=0.9467	p=0.1325^{ns} c=0.4286	p=0.7378^{ns} e=0.6282	p=0.68345^{ns} I_{50}=2.9819
15 °C	AG_3	0.9948	c=0	p=0.9714^{ns} d=0.9617	p=0.7476^{ns} c=1.3688	p=0.8962^{ns} e=20.305	p<0.00001*** I_{50sN}= 85.30 I_{50cN}= 2.71
20 °C	AG_3	0.802	c=0	p=0.445^{ns} d= 0.4248	p=0.5562^{ns} b=0.5953	p=0.9499^{ns} e=15.638	p=0.59856^{ns} I_{50}=0.1262
7 °C	PAC	0.8972	c=0	p=0.5646^{ns} d=1.07	p=0.7009^{ns} b=0.81638	e_{sN}=3.6273 e_{cN}=37.91952 p<0.0001****	p=0.38055^{ns} I_{50}=8.92966
15 °C	PAC	0.588	c=0	p=0.961^{ns} d=1.01125	p=0.3993^{ns} b=2.1005	p=0.111^{ns} e=0.34035	p=0.8381^{ns} I_{50}=1.5097

CONCLUSIONS

These results suggest that the sensitivity to gibberellic acid, or at least to GA_3, would not appear to be responsible for the germinative variations between both accessions.The effect of the maternal fertilization on seed dormancy and hence on germination, would be caused by differences in ABA:AG balance. However, it would be convenient to evaluate generations with a higher pressure of the evaluated factor (F_3, F_4) in order to clarify the tendency of a greater response by cN.

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