Spanish Journal of Agricultural Research (2005) 3(3), 331-334

Short communication. Seed germination of different populations of wild (n = 9) *Brassica montana* and *B. oleracea*

F. Pérez-García*

Departamento de Biología Vegetal. Escuela Universitaria de Ingeniería Técnica Agrícola. Universidad Politécnica de Madrid. Ciudad Universitaria s/n. 28040 Madrid. Spain

Abstract

The aim of this work was to evaluate seed germination characteristics of wild populations of *Brassica montana* (8 accessions) and *B. oleracea* (30 accessions). Seed germination at 25°C under a photoperiod of 16-h light/8-h darkness was variable in *B. montana* (0 to 86%) and more uniform in *B. oleracea* (73 to 100%). In both species, germination rate (as expressed by mean germination time) varied significantly among populations. A presowing treatment with gibberellic acid only significantly promoted germination in one of 14 populations tested from both species. The results of this study show high intraspecific variation in the germination pattern of seed from different *B. montana* populations and less variation in populations of *B. oleracea.*

Additional key words: final germination percentage, gibberellic acid, interpopulation variation, intraspecific variation, mean germination time.

Resumen

Nota corta. Germinación de semillas de diferentes poblaciones silvestres (n = 9) de *Brassica montana* y *B. oleracea*

El objetivo de este trabajo fue evaluar las características germinativas de semillas de poblaciones silvestres de *Brassica montana* (8 muestras) y *B. oleracea* (30 muestras). La germinación de las semillas a 25°C bajo un fotoperíodo de 16 h de luz/8 h de oscuridad fue variable en *B. montana* (0 a 86%) y más uniforme en *B. oleracea* (73 a 100%). En ambas especies, la velocidad de germinación (medida mediante el valor del tiempo medio de germinación) varió significativamente entre poblaciones. Un tratamiento previo a la siembra con ácido giberélico promovió significativamente la germinación en una sola de las 14 poblaciones ensayadas de ambas especies. Los resultados de este estudio muestran una elevada variación intraespecífica en el comportamiento germinativo de las semillas de las diferentes poblaciones de *B. montana* y una menor variación en las poblaciones de *B. oleracea.*

Palabras clave adicionales: ácido giberélico, porcentaje final de germinación, tiempo medio de germinación, variabilidad interpoblacional, variabilidad intraspecífica.

Brassica is the most important economic genus of the Brassicaceae. Several species of this genus have been cultivated from ancient times. Wild *Brassica montana* Pourret and *B. oleracea* L. are two species of the *B.oleracea* (n = 9) group which grow in Spain. These species are interfertile with each other and with cultivated *B. oleracea.* They can be an important source of genetic diversity for the breeding of cultivated *B. oleracea.* Both species are close related and grow in coastal habitats on vertical cliffs and on rocky slopes. In the Iberian Peninsula, *B. oleracea* grows on the Atlantic coast of North Spain and *B. montana* on the Mediterranean coast of Northeast Spain (Gómez-Campo *et al.,* 1999).

Several papers have reported work on the phylogenetic relationships among wild *Brassica* species with the chromosome number 2n = 18 (Lannér, 1998; Gómez-Campo *et al.,* 1999). However, we are not aware of any studies on the seed germination of wild populations of *B. montana* and *B. oleracea.* Seed germination is a major aspect of germplasm that needs to be understood when an increasing number of

* Corresponding author: felix.perez@upm.es
Received: 05-04-05; Accepted: 01-06-05.

samples of different origins are cultivated for experimental, breeding or commercial purposes (Bewley and Black, 1994). The objective of this work was to compare the germination responses (final germination percentage and mean germination time) of seeds from 8 populations of *B. montana* and 30 populations of wild *B. oleracea* under controlled germination conditions.

Brassica montana seeds were collected from 8 locations on the Mediterranean coast of Northeast Spain in July 2003. Wild *B. oleracea* seeds were collected from 30 locations on the Atlantic coast of North Spain in July 2002 and July 2003. Tables 1 and 2 show the collection site of each seed population. Seeds from all populations were equally ripe based on their colour and consistency. Seed was stored in a dry room at room temperature (ca. 23°C) until the start of germination trials in the autumn of the year that the seed was collected. Four replicates of 25 seeds each were used in each trial. Seeds samples were placed in a 7-cm glass Petri dishes on two sheets of filter paper moistened with 3.5 ml of distilled water. The water was replaced regularly. Petri dishes were incubated at 25°C with a 16-h light/8-h dark photoperiod. Cool white fluorescent tubes provided lighting with an irradiance of 35 μmol m^{-2} s^{-1}. Seeds with an emerged radicle were counted daily and removed from the Petri dishes. The final germination percentage was scored after a 30-day incubation.

Application of gibberellic acid (GA_3) was by soaking the seed in a GA_3 water solution (1000 mg l^{-1}) for 24 h prior to placing them in the germination cabinets. At the end of the germination period, the final germination percentage and the mean germination time (MGT, days) were calculated. The latter was determined according to the following formula (Bewley and Black, 1994):

$$\mathrm{MGT} = \Sigma T_i N_i / \Sigma N_i$$

where T_i is the number of days from the date of sowing and N_i is the number of seeds germinated at each day. The MGT was not calculated when the final germination was equal to or less than 5%. For all the tests the final germination percentages (mean values ± standard error) were calculated and arcsine transformed values were subjected to analysis of variance. For each accession, a one-way factorial ANOVA was performed. The statistical analysis of MGT was also carried out using one-way ANOVA.

Table 1 shows the final germination percentage and the MGT for untreated seed and seed soaked in GA_3 for all populations of *B. montana.* There were highly significant differences ($P < 0.001$) in the final germination among the different populations. The final germination of control seed ranged from 0 to 86%. The MGT also varied significantly ($P < 0.01$) among the different populations and ranged from 3.63 to 6.08 d.

Table 1. Final germination percentage (mean value ± standard error) and mean germination time (MGT, days ± standard error) of eight *Brassica montana* accessions. All seed collection sites are on the Gerona coast (NE Spain). Seeds were collected in July 2003. Seeds were germinated at 25°C under a 16-h light photoperiod. When germination was ≤ 5%, MGT was not calculated (NC).

Number[1]	Seed collection site	Germination (% ± SE)			MGT (days ± SE)		
		Control	GA_3	*P*	Control	GA_3	*P*
9855	Cap Norfeu	71 ± 1.66	80 ± 6.14	ns	3.63 ± 0.21	3.15 ± 0.25	ns
9856	Cap S. Sebastiá	86 ± 6.40	95 ± 2.28	ns	3.85 ± 0.13	3.60 ± 0.22	ns
9859	Punta d'en Bosc	56 ± 3.74	48 ± 6.63	ns	6.08 ± 0.25	5.32 ± 0.34	ns
9860	Cala Joana	45 ± 5.17	43 ± 2.69	ns	5.45 ± 0.66	5.05 ± 0.38	ns
9862	Cala Sr. Ramón	0 ± 0.00	2 ± 1.45	ns	—	NC	—
9863	Vallpresona	47 ± 3.28	65 ± 8.99	ns	4.68 ± 0.49	5.50 ± 0.14	ns
9864	Cala Salyons	1 ± 0.87	5 ± 0.87	*	NC	NC	—
9865	Cala Futadera	44 ± 6.48	53 ± 2.16	ns	3.83 ± 0.41	3.75 ± 0.37	ns

[1] The number correspond to the accession number in the germplasm bank of the Universidad Politécnica de Madrid (Spain). Control: seeds were incubated in distilled water (untreated seeds). GA_3: seeds were incubated in a gibberellic acid water solution of 1000 mg l^{-1} (treated seeds). * Significantly different at $P < 0.05$; ns, not significant.

Soaking with gibberellic acid (GA_3) only significantly ($P < 0.05$) enhanced germination in one of the eight accessions (Table 1). The germination rate as measured by the MGT was similar in control and GA_3 treated seed.

Table 2 shows the final germination percentage and MGT for control and treated seeds from all *B. oleracea* populations. There were significant differences ($P < 0.001$) in final germination among the different populations. However, in all populations the final germination of control seed was > 70% and ranged from 73 to 100%. Germination was ≥ 90% in 23 of the 30 accessions. The MGT values also varied significantly ($P < 0.001$) from 1.35 to 3.70 days depending on the population. Soaking seed in GA_3 did not significantly increase final germination ($P > 0.05$) over that of control seed in the six accessions that were assayed (Table 2). The MGT values of seed treated with GA_3 were significantly ($P < 0.05$ and $P < 0.01$) lower than in control seed in five of the six tested accessions.

Interpopulation differences in final germination were highly significant in *B. montana.* Populations from similar

Table 2. Final germination percentage (mean value ± standard error) and mean germination time (MGT, days ± standard error) of 30 *Brassica oleracea* accessions. Seed of accessions 9801 to 9831 was collected in July 2002, seed of accession number 9887 was collected in July 2003. Seeds were germinated at 25°C under a 16-h light photoperiod.

Number[1]	Seed collection site (Province of Spain)	Germination (% ± SE)			MGT (days ± SE)		
		Control	GA_3	*P*	Control	GA_3	*P*
9801	Cabo Vidio (Asturias)	97 ± 0.87	NO	—	1.35 ± 0.06	NO	—
9802	Cudillero (Asturias)	73 ± 3.57	76 ± 6.16	ns	3.05 ± 0.31	1.70 ± 0.16	*
9804	Playa de Xagó (Asturias)	79 ± 3.28	86 ± 3.00	ns	2.50 ± 0.23	1.46 ± 0.10	*
9805	Playa Negra (Asturias)	98 ± 1.73	NO	—	2.50 ± 0.15	NO	—
9806	Molín (Asturias)	96 ± 1.41	NO	—	3.10 ± 0.09	NO	—
9807	Cabo Peñas (Asturias)	90 ± 3.32	NO	—	1.77 ± 0.10	NO	—
9808	Playas de Viodo (Asturias)	100 ± 0.00	NO	—	2.22 ± 0.10	NO	—
9809	Llumeres (Asturias)	96 ± 2.45	NO	—	2.70 ± 0.11	NO	—
9810	Candas (Asturias)	97 ± 1.66	NO	—	2.05 ± 0.07	NO	—
9811	Gijón (Asturias)	73 ± 2.96	69 ± 2.81	ns	3.70 ± 0.21	1.91 ± 0.32	*
9812	Tazones (Asturias)	99 ± 0.87	NO	—	1.62 ± 0.07	NO	—
9813	Playa de San Antolín (Asturias)	80 ± 2.00	89 ± 3.84	ns	2.42 ± 0.06	2.40 ± 0.38	ns
9814	Niembro (Asturias)	94 ± 3.00	NO	—	2.32 ± 0.06	NO	—
9815	Puerto de Llanes (Asturias)	98 ± 1.00	NO	—	1.37 ± 0.06	NO	—
9816	Pendueles (Asturias)	89 ± 2.18	NO	—	2.10 ± 0.03	NO	—
9817	La Franca (Asturias)	98 ± 1.00	NO	—	3.02 ± 0.14	NO	—
9818	Pimiango (Asturias)	96 ± 1.41	NO	—	2.65 ± 0.11	NO	—
9819	Oyambre (Cantabria)	95 ± 1.66	NO	—	1.97 ± 0.12	NO	—
9820	Laredo (Cantabria)	76 ± 5.10	88 ± 2.45	ns	2.60 ± 0.08	1.56 ± 0.16	**
9821	Marrón (Cantabria)	97 ± 1.66	NO	—	2.02 ± 0.02	NO	—
9823	San Juan de Gaztelugatxe (Vizcaya)	95 ± 0.87	NO	—	2.08 ± 0.04	NO	—
9824	Bermeo (Vizcaya)	97 ± 1.66	NO	—	2.26 ± 0.04	NO	—
9825	Lekeitio (Vizcaya)	90 ± 1.00	NO	—	2.27 ± 0.11	NO	—
9826	Ondarroa (Vizcaya)	94 ± 1.73	NO	—	2.17 ± 0.06	NO	—
9827	Guetaria (Guipúzcoa)	98 ± 1.73	NO	—	2.00 ± 0.00	NO	—
9828	Puerto de Guetaria (Guipúzcoa)	93 ± 2.96	NO	—	2.17 ± 0.04	NO	—
9829	Monte Igueldo (Guipúzcoa)	79 ± 6.06	88 ± 1.41	ns	3.40 ± 0.39	1.35 ± 0.13	**
9830	Monte Urgull (Guipúzcoa)	93 ± 0.87	NO	—	2.13 ± 0.05	NO	—
9831	Monte Ulía (Guipúzcoa)	94 ± 1.73	NO	—	2.16 ± 0.11	NO	—
9887	Cuchía (Cantabria)	92 ± 2.45	NO	—	2.18 ± 0.05	NO	—

[1] The number correspond to the accession number in the germplasm bank of the Universidad Politécnica de Madrid (Spain). Control: seeds were incubated in distilled water (untreated seeds). GA_3: seeds were incubated in a gibberellic acid water solution of 1000 mg l^{-1} (treated seeds). When the germination of control seeds was > than 80% a GA_3 trial was not carried-out (NO). ns, not significant. * Significantly different at $P < 0.05$. ** $P < 0.01$.

habitats may have different germination responses. These differences can arise from environmental variation during seed maturation and the effect of maternal genotype (Fenner, 1991; Wulff, 1995). Variability of germination response within populations of a species is frequent in wild Mediterranean species (Pérez-García *et al.,* 2003). Indeed, interpopulation variability in seed germination may be interpreted as an important survival strategy of species growing under variable and unpredictable environmental conditions (Gutterman, 1994; Kigel, 1995; Baskin and Baskin, 1998; Cruz *et al.,* 2003).

Less interpopulation difference was found in seed germination of *B. oleracea.* Also, the germination was high in all populations (> 70%). The germination of the different *B. oleracea* populations was much more uniform that of the *B. montana* populations.

Interpopulation variability in the germination of *B. montana* might have arisen as an adaptation strategy to the more unpredictable habitats where this species grow on Mediterranean coast. On the Atlantic coast of Spain there is frequent rain, even in summer, and temperatures are not extreme. On the Mediterranean coast the summer are usually very dry with high temperatures. The high variability in germination shown by the different *B. montana* populations could be explained by the stress conditions of high temperature and drought which are characteristics of a Mediterranean summer.

Gibberellic acid (GA_3) only significantly enhanced germination in one of the eight accessions of *B. montana* and in none of the six accessions of *B. oleracea* which were tested. The MGT of GA_3 treated seeds in both species was higher than in control seeds. Moreover, these differences were significant in five of the six *B. oleracea* accessions which were tested. These results suggest the presence of a low level of seed dormancy in both species.

In conclusion, the results of this study show high intraspecific variation in the germination pattern of seed from different *B. montana* populations (Mediterranean coast species) and less variation in populations of *B. oleracea* (Atlantic coast species). Therefore, future studies of the germination characteristics of *B. montana* should consider different populations.

Acknowledgements

To the Ministry of Science and Technology (Spain) and the National Institute for Agricultural and Food Research and Technology (INIA) Project RF01-016 who financed this research.

References

BASKIN C.C., BASKIN J.M., 1998. Seeds ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego.

BEWLEY J.D., BLACK M., 1994. Seeds: physiology of development and germination. Plenum Press, NY.

CRUZ A., PÉREZ B., VELASCO A., MORENO J.M., 2003. Variability in seed germination at the interpopulation and intraindividual levels of shrub *Erica australis* in response to fire-related cues. Plant Ecol 169, 93-103.

FENNER M., 1991. The effects of the parent environment on seed germinability. Seed Sci Res 1, 75-84.

GÓMEZ-CAMPO C., TORTOSA M.E., TEWARI I., TEWARI J.P., 1999. Epicuticular wax columns in cultivated *Brassica* species and their close wild relatives. Ann Bot 83, 515-519.

GUTTERMAN Y., 1994. Strategies of seed dispersal and germination in plants inhabiting deserts. Bot Rev 60, 373-425.

KIGEL J., 1995. Seed germination in arid and semiarid regions. In: Seed development and germination (Kigel J. and Galili G., eds). Marcel Dekker, NY. pp. 645-699.

LANNÉR C., 1998. Relationships of wild *Brassica* species with chromosome number 2n=18, based on comparison of the DNA sequence of the chloroplast intergenic region between *trnL* (UAA) and *trnF* (GAA). Can J Bot 76, 228-237.

PÉREZ-GARCÍA F., HORNERO J., GONZÁLEZ-BENITO M.E., 2003. Interpopulation variation in seed germination of five Mediterranean Labiatae shrubby species. Israel J Plant Sci 51, 117-124.

WULFF R.D., 1995. Environmental maternal effects and seed quality and germination. In: Seed development and germination (Kigel J. and Galili G., eds.). Marcel Dekker, NY. pp. 491-505.