Sunflower (*Helianthus annuus* L.) seed dormancy period: a comparison between two hybrids

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ABSTRACT

- Sunflower seed dormancy begins around the third week after pollination and is overcome during storage. It is induced by abscisic acid and broken by ethylene. Dormancy period depends on the genotype and the environmental conditions during maturation and storage. The aims of the present study were to compare two hybrids dormancy period and to understand the causes of the differences between them.

- The hybrids H1020 and H3040 and their parental lines (B10 and R20, B30 and R40, respectively) were obtained in 2004/05 in Argentina at two locations: Baigorrita and Ballenera. In Ballenera, two other crosses A10 X R40 (H1040) and A30 X R20 (H3020) were also obtained. H1020 and H3040 naked embryos were treated with abscisic acid, Ethephon and 1-Aminocyclopropane-1-carboxylic acid. The experimental design was randomized with 3 replications. Each replication consisted of 25 embryos (N= 3150) and the germination temperature was 16°C. Analysis of variance and Tukey's test were performed on the data. After harvest, germination capacity of all the materials was evaluated weekly until achenes reached 85% of germination. This experiment consisted of 6 replications of 50 achenes each (N= 4200).

- Seed produced in Baigorrita, under higher temperature and less humid conditions, had a shorter dormancy period (except for R40). H1020 and H3040 produced in Baigorrita showed similar dormancy period, while in Ballenera H1020 needed 17 days more than H3040 to reach 85% of germination. These results suggest that H1020 is more affected by environmental conditions showing a longer dormancy period than H3040. Dormancy release pattern of parental lines indicated that the longer dormancy period showed by H1020 produced in Ballenera could be inherited from R20. Growth regulator experiments showed that H1020 is more sensitive to abscisic acid than H3040. H1020 longer dormancy could be explained by its higher sensitivity to this growth regulator.

- The evaluated genotypes showed different seed dormancy behavior. R20 inbred line genetic background carry alleles increasing this period. With respect to environment the best conditions in order to reduce the dormancy period were high air temperature and low relative humidity.

- This study confirms the existence of genetic variability for the dormancy period in this species. The present results will be useful when breeding for this trait.

Key words: abscisic acid – ethylene – dormancy – embryos - achenes

INTRODUCTION

Dormancy is a process that avoids seeds germination even though they are viable and provided with optimum conditions. (Moreira de Carvalho and Nakagawua, 1988). Sunflower incapacity to germinate arises from both embryo dormancy, especially important at temperatures below 25°C, and a seed-coat imposed dormancy, more important at temperatures between 25 to 40°C. This inability is established around the third week after pollination and it is gradually eliminated during dry storage of the seeds (Corbineau, 1987).

Dormancy is induced by abscisic acid (ABA) (Le Page-Degivry et al., 1990) and broken by ethylene (Corbineau and Côme, 2003), possibly through protease activity (Borghetti et al., 2002). In that sense, it was found that germination of dormant sunflower seeds is strongly stimulated by the ethylene generating compound, 2-chloroethylphosphonic acid (Ethephon) (Kumar and Sastry, 1974, 1975; Harada, 1982) and by the immediate precursor of ethylene 1-aminocyclopropane-1-carboxylic acid (ACC) (Corbineau et al., 1990). Dormancy duration in sunflower is genetically controlled and genotypic variability has been reported (Subramanyam et al., 2002; Maiti et al., 2006). Environmental conditions during seed maturation and storage have also an influence on the dormancy period (Cseresnyes, 1978; Bazin et al., 2011).

Dormancy is a necessary attribute of sunflower crop because it prevents seed germination in the plant when harvest is delayed due to rainy or humid weather. However, presence of dormancy causes great problem if it is not overcome before the sowing in the following season. The aims of the present study were to compare the dormancy period of two hybrids and to understand the causes of the differences between them.

MATERIALS AND METHODS

The hybrids H1020 and H3040 and their parental lines (B10 and R20, B30 and R40, respectively) were obtained at two Argentinean locations: Baigorrita (35° S) and Ballenera (38° S), in 2004-2005. In Ballenera, the crosses A10 X R40 (H1040) and A30 X R20 (H3020) were also obtained. In order to evaluate the sensitivity of hybrids H1020 and H3040 to the growth regulators involved in seed dormancy, the following treatments were conducted before harvest date with seed produced in Baigorrita:

(i) Thirteen days after pollination (13 DAP), when seeds were still able to germinate and exogenous ABA was effective (Le Page-Degivry, *et al*., 1993), seeds of H1020 and H3040 were rescued. The naked embryos were treated with Abscisic acid (Sigma®). The concentrations were: 0 (control), 5 X 10^{-9} M, 5 X 10^{-8} M, 5 X 10^{-7} M, 5 X 10^{-6} M, 5 X 10^{-5} M and 5 X 10^{-4} M.

(ii) A second rescue of both hybrids was done 34 DAP, when embryos were already dormant. These embryos were treated with Ethephon, (Ethrel 48 SL, from BAYER). The concentrations were: 0 (control), 5 ppm, 10 ppm, 15 ppm, 30 ppm, 60 ppm and 100 ppm.

(iii) The last rescue was done 45 DAP, and the embryos were treated with ACC (Sigma ®). The concentrations were 0 (control), 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M and 10^{-2} M.

The described treatments were applied to naked embryos (without pericarp or tegument) which were placed in plastic boxes containing a layer of cotton imbibed with deionized water or a growth regulator solution. The boxes were incubated in darkness and the germination temperature was 16°C. A naked embryo was considered to have germinated when the radicle had elongated 2-3 mm (Corbineau et al., 1990). The experimental design was randomized with 3 replications. Each replication consisted of 25 embryos (total number of embryos= 3150). Normality of the empirical distribution of each variable was assessed by the Shapiro-Wilk W test statistic. Homogeneity of variance was evaluated using the Levene's test on absolute residuals. Data were analyzed by analysis of variance and treatments means were compared using Tukey's multiple-comparison test. Statistical analyses were performed using R software, version 2.12.1 (R Development Core Team, 2010).

After physiological maturity all the inbred lines and hybrids from Baigorrita and Ballenera were harvested and stored at 15°C. The germination capacity of all materials was evaluated weekly. Achenes were placed in plastic boxes containing a layer of cotton imbibed with deionized water and incubated in darkness under 20°C temperature. This experiment consisted of 6 replications of 50 achenes each (N= 4200). A whole achene was considered to have germinated when the radicle had pierced the pericarp. The end of the dormancy period was recorded as the date when the samples reached 85% of germination.

RESULTS

Seed produced in Ballenera had a longer dormancy period than seed produced in Baigorrita, except for line R40 (Table 1). With regards to environmental conditions during seed maturation, in Baigorrita temperature was higher, while in Ballenera there were more rainy days and relative humidity (Table 2).

Table 1. Days between pollination and the end of the domancy period for all the genotypes obtained in Baigorrita and Ballenera

Location	Genotype							
	B10	R20	H1020	B30	R40	H3040	H1040	H3020
Baigorrita	94	104	94	94	71	94	-	-
Ballenera	106	149	119	115	71	102	98	115

Table 2. Environmental conditions during seed maturation in Baigorrita (Source: Aeroclub Junín, Buenos Aires) and Ballenera (Source: Experimental Field Miramar, Ministry of Agrarian Matters, Buenos Aires Government.

	Location	
	Baigorrita	Ballenera
Mean T°C	22.0	19.6
Minimum mean T°C	15.6	12.6
Maximum mean T°C	28.5	26.6
Rainy days	2	14
Total rain (mm)	86	84
Mean relative humidity (%)	69.4	73.1

The dormancy period for the hybrids H1020 and H3040 produced in Baigorrita was the same: both hybrids reached 85% of germination in 94 days. However, in Ballenera H1020 reached that value 17 days later than H3040 (Fig. 1a-b).

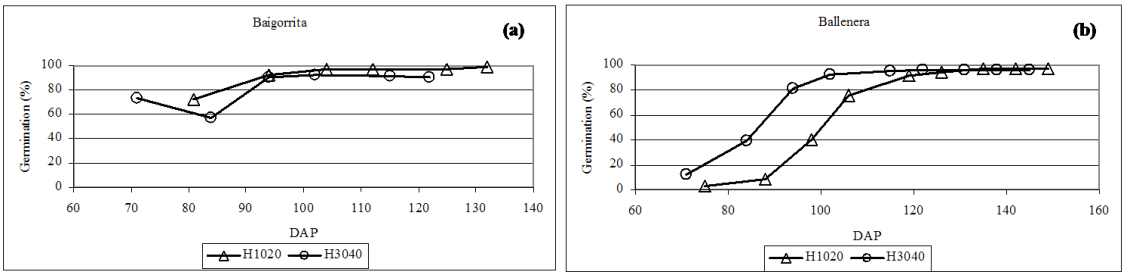

Fig. 1. Germination rate evolution of hybrids H1020 and H3040 produced in: **(a)** Baigorrita and **(b)** Ballenera. Each value in the plot is the mean germination rate of 6 replications of 50 achenes.

Dormancy release pattern of parental lines produced in Ballenera indicated that the longer dormancy period showed by H1020, could be inherited from the restorer line R20 (Fig. 2a). Dormancy period of this inbred line was 149 days, while for the other 3 inbred lines it ranged between 71 and 115 days. All hybrids from the R20 male parental line showed a longer dormancy period than those from R40 inbred (Fig. 2b)

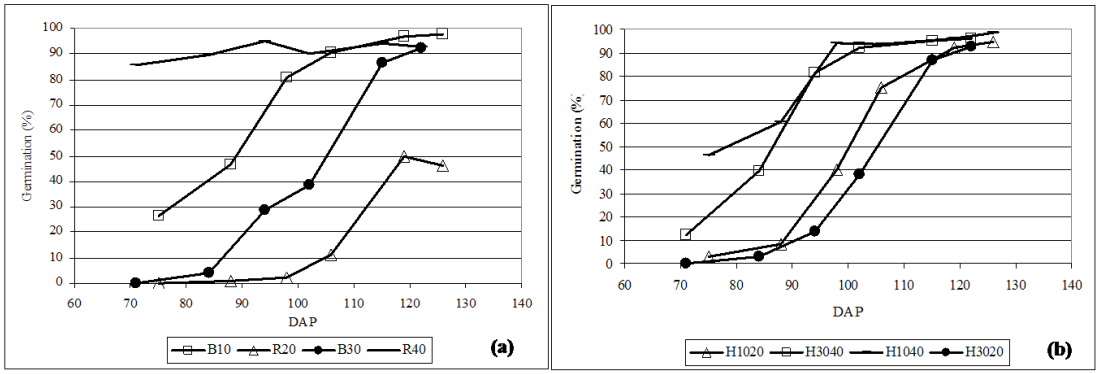

Fig. 2. Germination rate evolution of **(a)** parental inbred lines and **(b)** hybrids obtained in Ballenera. Each value in the plot is the mean germination percent of 6 replications of 50 achenes.

Results from the present study shows that ABA treatment caused a different effect on H1020 and H3040 non-dormant embryos. At the dilution 5 X 10^{-6} M H3040 percentage of germination remained high (89%), while at the same concentration, H1020 showed 52% of germinated embryos (Fig. 3a). Both hybrids displayed a similar pattern for Ethephon and ACC treatments (Fig 3b-c). Ethephon strongly stimulated germination of dormant embryos, while ACC had no or very little effect on dormancy of these materials.

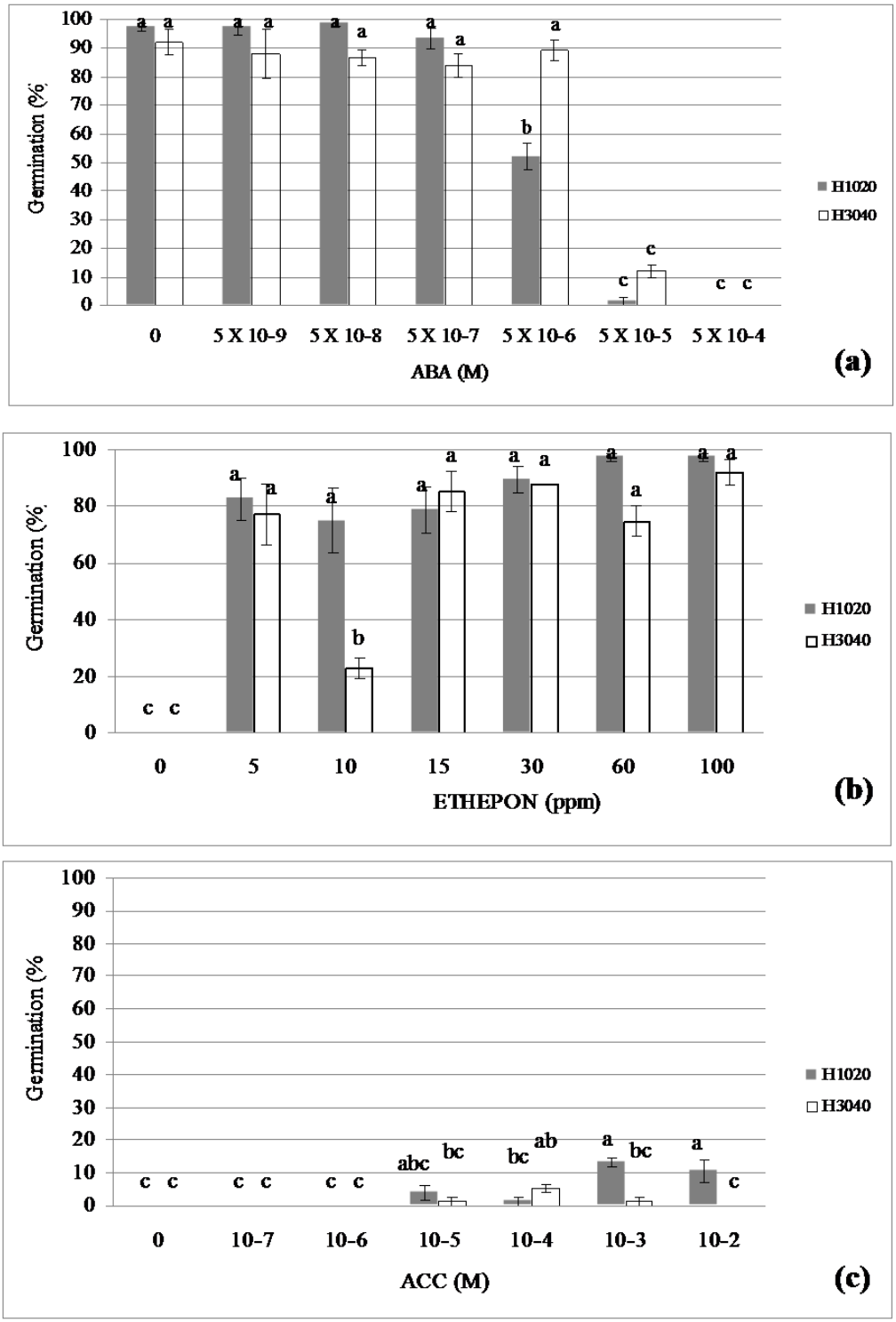

Fig. 3. Effect of growth regulators on the embryo germination rate: **(a)** Abscicic acid (ABA) **(b)** Ethephon and **(c)** 1-Aminocyclopropanecarboxylic acid (ACC). Each value in the plot is the mean of 3 replications of 25 embryos. Means with the different letter are significantly different (α=0.05)

DISCUSSION

The results from the present study suggest that the best conditions in order to reduce the dormancy period are high air temperature and low relative humidity and are in agreement with other authors (Fenner, 1991).

The germination rate evolution of hybrids H1020 and H3040 produced in Baigorrita and Ballenera demonstrates that the environment did not affect both genotypes equally. When produced in Ballenera, hybrid H1020 showed a longer dormancy period than H3040 (the difference between these hybrids was 17 days). This difference could be explained by the higher sensitivity of H1020 to ABA, since at 5×10^{-6} M concentration H3040 germination percentage remained high (89 %), while at the same concentration H1020 performed 52%.

The dormancy release pattern of parental lines and hybrids produced in Ballenera showed that all hybrids from the R20 male performed a longer dormancy period suggesting that this characteristic is genetically controlled.

In accordance with previous results the growth regulators experiments showed that Ethephon strongly stimulated germination of dormant embryos (Corbineau and Côme, 2003). Conversely ACC had no or very little effect on dormancy of these materials. This result does not agree with Corbineau et al. (1990) who found that ACC was an effective treatment for breaking embryo dormancy**.**

The present results confirmed the existence of genetic variability for the dormancy period and showed that R20 inbred line genetic background has alleles that increase this period.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Françoise Corbineau, Dr. André Merrien, Dr. Brady Vick, Dr. Dragan Skoric and Dr. Gary Fick. for their important advices and help .

They are also thankful to Susana Marenchino and to NIDERA Baigorrita team for their collaboration and to Ing. Agr. *MSc* Graciela Nestares for her inputs, discussion of ideas and critical review of the present work.

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