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STORAGE TIME ON SEED DORMANCY AND GERMINATION IN *ETI MUTANTS* OF *ARABIDOPSIS THALIANA* (L) HEYNH

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Abstract: Previous studies have reported significant influence of maternal environment on dormancy and germination of seeds. We investigated the dormancy level and germinability in seeds obtained from mutants of *Arabidopsis thaliana* grown under long-day and short-day photoperiodic conditions. Seed dormancy and germination responses were significantly influenced by photoperiod, genotype and storage time. Strong interactions between the main factors imply that the actual pattern of dormancy and germination responses to each factor depends on the levels of other factors. The maternal environment has greater influence on dormancy than that of genotype. However, it appears that there is an overriding effect of storage period in determining levels of dormancy. Duration of the storage period had marked effect on the germination properties of the seeds that are exacerbated by the genetic variation. The genetic and non-genetic effects disappeared when the seeds were exposed to red light after stratification, implying the transitory nature of maternal influence on seed dormancy and germination characteristics.

Keywords: Arabidopsis, eti mutants, maternal environment, storage period, seed dormancy.

INTRODUCTION

Maternal effects can be divided into genetic and non-genetic effects. The former includes genetic differences among mother plants that results in differences in the provisioning or other aspects of the environment of seeds as they develop within flower and fruits. Genetic maternal effects can occur due to the genetic material in mitochondria, chloroplast or plastids, which are contributed only by mother [Roach and Wulf 1987, Platenkamp and Shaw 1993, Weiner *et al*. 1997]. Non-genetic maternal effects can occur because the environment in which mother plant grows may influence her ability to provision seeds. For example, the seed nutrient content can be influenced by the soil nutrient level in which the mother plant is growing [Parrish and Bazzaz 1985].

Environmental maternal effects in plants often appear to be transitory [Miao *et al*. 1991, Schmid and Dolt 1994], but they could still play a role if the period of their influence is important for plant fitness, e.g. germination time [Alexander and Wulff 1985, Platenkamp and Shaw 1993]. In most commonly described scenario, environmental maternal effects are mediated by seed characteristics, and there are several studies showing a positive relationship between seed traits and speed of germination and subsequent seedling size [Crawley and Nachapong 1985, Schmid and Dolt 1994, Weiner *et al*. 1997]. The studies of Parrish and Bazzaz [1985],

Roach and Wulff [1987] demonstrated that the environment experienced by the mother plant before seed dispersal can have large effects on the germination of the seed produced. Bennington *et al.* [1991] and Orozoco-Segovia *et al.* [1993] made similar comments. Although the germination characteristics of a seed will determine the degree and type of dormancy in that seed, the germination pattern observed will be influence by differences in environmental conditions during the course of development. Gutterman [1982] provides an excellent review of some of the ways in which these maternal effects determine the germination behavior of mature seeds. One of the most elegant studies of the maternal effect on seed during their development is that of Cresswell and Grime [1981]. They showed that the light requirement for germination observed in many herbaceous plants is imposed during the course of maturation by the light-filtering properties of maternal tissue that surround the developing seeds. If the structures investing the seeds remain green throughout the maturation of the seeds, light requirements for germination will be induced in seeds before they shed. This is thought to be because the phytochromes in seed are arrested in the inactive form (P_r) and the light stimulus which is required to convert P_r to the active (P_{fr}) form that allows germination to proceeds, is excluded by the outer seed structures. Seeds of many species mature while the surrounding tissues are still green, and thus embryos are in a far-red light environment. Such seeds are dormant, whereas those whose covering tissues have little chlorophyll are often non-dormant when mature. Thus the differences in depth of dormancy are brought about, at least in part, by the genetic make-up, the environmental conditions in which the plants are grown and the nature of the seed covering structures.

The conditions of illumination and the photoperiod experienced by the parent plant, especially during the last few days of seed maturation, clearly affect dormancy in certain species. For example, *Chenopodium album* has deeply dormant seeds when the fecund plants are held under long days but non-dormant seeds are produced under short days. A similar response to day-length has been reported in *Trigonella arabica* and *Partulaca aleracea* [Gutterman 1982]. However, the opposite relationship between day-length and dormancy occurred in *Polygonum monspeliensis*. In some cases differences in photoperiod can be correlated with permeability, thickness and color that may contribute to impermeability and light-filtering properties of the seed coat [Fenner 1985]. In other cases it is embryo where photoperiod may influence depth of dormancy.

Therefore many factors create the complex and dynamic light environment in which seed develop. Few studies have tried to elucidate the complex optical properties of plant tissue [Vogelmann 1986]. Identification of the environmental factors responsible for modifications in seed dormancy is essential for deeper understanding of reproductive

strategies of plants. The “maternal environmental” approach has proved useful for studying dormancy mechanism in seeds of *Chenopodium album* [Karssen 1970], *Datura ferox* [Sanchez *et al*. 1981], *Sorghum halepense* [Benech *et al.* 1988], *Centaurea maculosa* [Weiner *et al*. 1997], forest trees [Kyereh *et al*. 1999], *Atriplex sagittata* [Mandak and Pysek 2001].

INFLUENCE OF DRY STORAGE ON DORMANCY PATTERNS

It is quite common for seeds to be dormant when they are fully mature on the mother plant. But it gradually becomes non dormant during dry storage. This type of dormancy that is described as due to the need for “after-ripening in dry storage” is evidently due to some causes within the seed itself and disappears spontaneously with time. However the length of the storage time required varies from species to species. It is often suggested that dry storage alter the properties of seed coat and the endogenous hormonal level so that germination becomes possible [Khan 1971].

The aim of this work is to answer the following questions:

1. Does photoperiod to which the mother plant is exposed during seed germination affect dormancy level in the resultant seeds?
2. Does the influence of maternal environment on dormancy level of resultant seeds decrease over the periods of dry storage?
3. Does the environmental maternal effect override that of genetic maternal effects?

MATERIALS AND METHODS

The seeds used were the mutants obtained from the wild type population of *Arabidopsis thaliana* (L.) by chemical mutagenesis using ethyl-methane-sulphonate [Harpham *et al*. 1991]. Seeds of six genotype: *Wild type, eti* 3*, eti* 5*, eti* 8*, eti* 10*, eti* 13, were raised under uniform conditions on the surface of damp Levington`s Seed Compost (No. 1) in 35 x 32 cm seed trays, covered by glass to maintain humidity in a glasshouse (25 ^{o}C max., 15 ^{o}C min). Supplementary lighting being supplied by tungsten bulbs ensured 16 hours photoperiod. The glass was removed after one week when the cotyledons spread apart and the first leaf was just visible. The plants were then shifted to controlled-environment Saxcil growth chambers that provide PAR of 89? mol m^{-2} s^{-1}. A short day-length, 8 hour, was given to one group of plants and was designated as short-day treatment. For the second group of plants, a long day-length, 16 hours, was chosen for the long day treatment. The trays were arranged randomly in the growth chambers, kept well watered and periodically rotated to minimize possible positional effects in the chamber. The arrangement was made to ensure that plants in each tray received similar experimental level of radiation with minimal shading by plants in adjacent trays.

Only completely mature siliquae were harvested and were air dried at 24 ± 2 °C. Three to four days after drying, the seed collections were cleaned and kept in individual envelopes labelled accordingly with type of mother plant and date of harvest. Seeds were stored at 24 ± 2 °C in darkness in a desiccated atmosphere in order to record further changes in germination during dry storage.

Germination trails of *wild type* and mutant seeds were conducted monthly using seeds from both light treatments. All germination studies were conducted with 30 seeds per replicate and with five replicates in each treatment. Seeds were incubated in plastic petri dishes on the surface of two Whatman No. 1 filter papers moistened with 1ml of sterile distilled water. The germinated seeds were counted and removed after six days. Visible radical protrusion was the criterion for germination. At the end of the experiment, un-germinated seeds in each dish were evaluated for viability. Seed viability was determined by using the tetrazolium test (TZ). Combined effect of red light and stratification was also investigated.

DATA ANALYSIS

The percentage data were arc-sin transformed and evaluated using analysis of variance. Interactions between treatments and genotypes are presented as graphs with significant differences indicated by bars of least significant range, derived from Duncan's Multiple Range Tests at $P < 0.05$.

RESULTS

Tables 1 and 2 show the analysis of variance (ANOVA) and results of the germination data. Despite the complexity of the interactions, the ANOVA demonstrated that much of the variations in germination properties could be explained by differences in photoperiod, genotype and duration of the storage time. Storage time was more important of the two variables when considered alone ($F = 366.05$, $P < 0.001$). But there was also a significant first order interaction ($F = 26.57$, $P < 0.001$) between genotype and photoperiod suggesting that the effect of maternal environment cannot be separated from the genotype. Examination of the data from both photoperiods demonstrate that, with the exception of *eti* 5, when comparison are made within the same genotype, mean values for long day (LD) were generally higher than those of short day (SD). These trends indicate that there were two sources of dormancy: one inherited to the seeds themselves (Innate dormancy), and superimposed on these values is the influence of the maternal environment during seed development (imposed dormancy).

Duration of the storage period had a marked effect on the germination properties of both seed lots. The significant interactions between P x T and G x T (Table 1) indicated that the time course variations in germination properties of the two seed lots were influenced by the genetic

factor. Comparison between the two seed lots (Fig.1) shows that LD seeds exhibited higher germination percentages than SD and both the seed lots showed an initial rise followed by a gradual fall.

Table 1. Analysis of variance of germination data obtained from *wild type* and five *eti* mutants of *A. thaliana* seeds. Seeds were obtained from plants grown under two different photoperiods.

Source of Variation	Degree of freedom	Mean squares	*F*-RATIO
Photoperiod (P)	1	20365.559	257.63***
Genotype (G)	5	8762.482	110.85***
Storage time (T)	6	28936.279	366.05***
P x G	5	2100.040	26.57***
P x T	6	992.627	12.56***
G x T	30	840.630	10.63***
P x G x T	30	555.013	7.02***

- P <0.05. ** P <0.01, ***P <0.001.

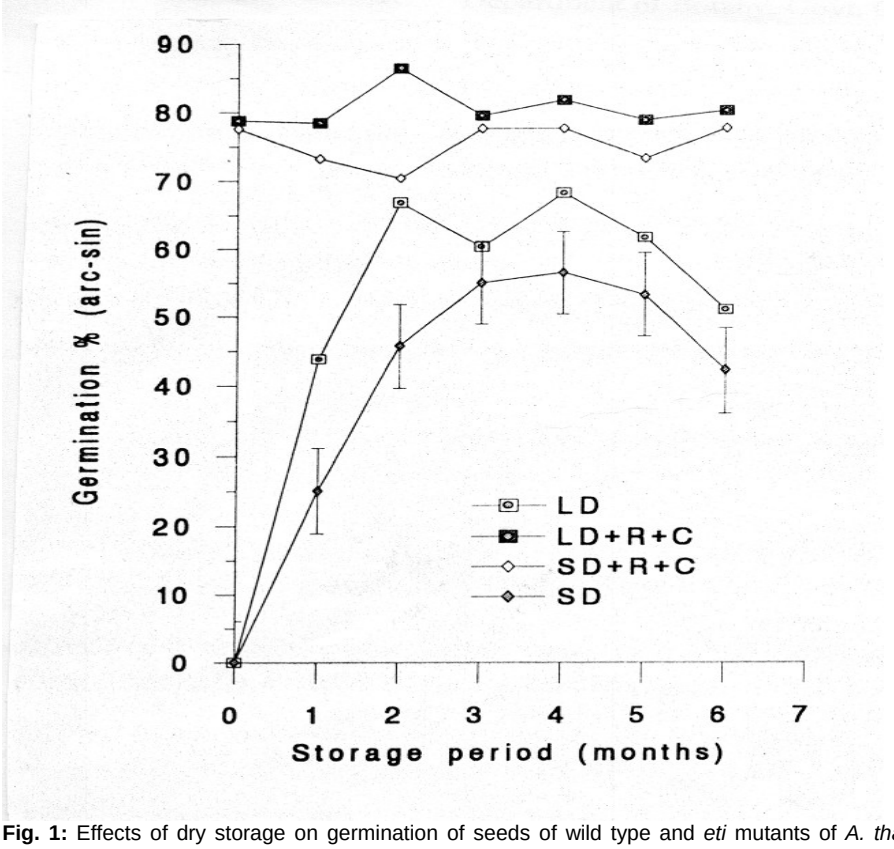

Fig. 1: Effects of dry storage on germination of seeds of wild type and *eti* mutants of *A. thaliana*. Seeds were collected from the plants grown under short day (SD) and long day (LD) photoperiod. Germination was observed with and without exposure to 18h red light (R) plus 3-days stratification (C)

Results depicted in Fig. 1 show that time to which maximum germination occurs was delayed by one month in SD seeds. Wild type seeds exhibited the maximum differences between two seed lots after two months of dry storage and this difference was due to delayed removal of primary

dormancy in the SD seed compared to LD (Table 2). Unlike wild type, data for *eti* 8 suggested that the differences between the two lots occurred due to the imposition of secondary dormancy in the SD seed after six months storage period. *eti* 3, *eti* 5, *eti* 13 showed less variability between the two lots than the former genotypes, indicating less sensitivity to the maternal environment in terms of dormancy. The pattern shown by eti 10 was rather different.

Table 2: Mean germination percentage (n = 4) during six month dry storage of two seed lots: collected from plants of *A. thaliana* grown under long day (LD) and short day (SD) conditions . Seeds were stored at 24°C during the experiment. Germination was noted after six days incubation in dark at 24 °C. Values that are not significantly different at P = 0.05 have the same superscript letters.

Seed lot	Storage period (Months)							
	0	1	2	3	4	5	6	Mean
				Wild type				
LD	00.00	70.00^{c}	100.00^{d}	97.50^{d}	100.00^{d}	97.50^{d}	47.50^{b}	73.21
SD	00.00^{a}	00.00^{a}	15.00^{b}	85.50^{e}	85.00^{e}	47.50^{d}	37.50^{c}	38.21
				Eti 3				
LD	00.00^{a}	52.00^{b}	87.50^{c}	97.50^{cd}	92.50^{c}	92.50^{c}	92.50	73.50
SD	00.00^{a}	35.00^{b}	97.50^{e}	100.00^{e}	82.50^{d}	70.00^{c}	82.50^{d}	66.79
				eti 5				
LD	00.00^{a}	15.00^{ab}	47.50^{d}	50.50^{d}	25.00^{c}	10.00^{ab}	7.50^{a}	22.14
SD	00.00^{a}	00.00^{a}	17.50^{b}	17.50^{b}	30.00^{c}	50.00^{d}	27.50^{c}	20.36
				eti 8				
LD	00.00^{a}	40.00^{b}	100.00^{cd}	82.50^{c}	100.00^{cd}	100.00^{cd}	95.00^{c}	73.93
SD	00.00^{a}	00.00^{a}	52.50^{c}	62.50^{d}	82.50^{f}	77.50^{e}	22.50^{b}	42.57
				eti 10				
LD	00.00^{a}	45.00^{b}	92.50^{c}	85.00^{c}	100.00^{de}	85.00^{c}	95.00^{cd}	71.79
SD	00.00^{a}	37.50^{b}	47.50^{c}	52.50^{c}	57.50^{cd}	67.50^{def}	65.00^{de}	46.79
				eti 13				
LD	00.00^{a}	65.00^{c}	80.00^{d}	90.00^{e}	100.00^{f}	80.00^{d}	27.50^{b}	63.21
SD	00.00^{a}	35.00^{b}	77.50^{c}	87.50^{cd}	80.00^{c}	72.50^{c}	35.00^{b}	55.31

The primary dormancy in both the seed lots changed more or less in parallel fashion and was not much modified with time, although the SD showed significantly less germination than the SD. This difference became more pronounced as the experiment progressed. All the differences between the seed lots and genotypes appeared under dark conditions became masked when the seeds were exposed to 18 hours red light after three days stratification at 4 °C (Fig. 1).

DISCUSSION

There were large and highly significant differences in the mean germination of seeds produced by plants grown under LD and SD conditions. This response indicates that exposing of the mother plants to different environment could significantly affect the germinability of the resulting seeds. These results are in agreement with those of Hayes and Klein [1974], Gutterman [1980], Benech *et al.* [1988] and Weiner *et al*. [1997]. Not only the maternal environment, but also the storage period has huge effect on dormancy and germination pattern of seeds. Similar results have also been reported by Bennington *et al*. [1991] using seeds

of *Luzula parviflora*. The depression in seed germination found in seed from SD collections in the dark might be explained in terms of relative longevity of the chlorophyll rich maternal structures investing the seeds, which is considered to be important in determining the fate of seeds to germinate in the dark [Cresswell and Grime 1981]. In fact chlorophyll of the investing tissue will absorb red light but not wavelengths longer than about 710 nm, hence the transmitted light, being rich in the far-red component, served to lower the amount of P_{fr} and thus the embryos remained in a far-red rich environment for an extended period during seed development. This low percentage germination response to the photoperiod to which the mother plant is exposed is what Gutterman [1982] terms "photoperiodic or chlorophyll screening dormancy". This phenomenon also occurs in nature, where the source of far-red light is filtered through green tissues [Bewley and Black 1985].

Inter-genotypic variation found in response to photoperiod is presumably genetic in origin, reflecting the differences in light filtering properties or light perception capacity [Grime, 1966].

The freshly harvested seeds of both the lots were dormant when placed in the dark at 24 ^{o}C but gradually became non-dormant with increased dry storage. These trends indicate that dormancy disappeared spontaneously with time. The period required for maximum germination varies considerably between the two seed lots and also between the genotypes. Barton [1965], Wareing [1965], Fenner [1985], Singh and Amritphale [1991] reported similar results.

The germination study carried out in the dark at 24^{o}C exhibit large differences between the SD and LD seed lots particularly during the first two months post harvest. After six month, the magnitude of these differences is decreased. The environmental maternal effects are usually weak or transitory [Alexander and Wulff 1985, Schmitt *et al*. 1992, Platenkamp and Shaw 1993, Wulff *et al.* 1994, Sultan 1996]. Although environmental maternal effects are detectable, they are quite weak compared with many of the other influences in determining the levels of dormancy, such as storage period. Furthermore, these trends indicate that imposed dormancy is a rather plastic trait and with subsequent manipulation, maternal differences decline with time [Roach and Wulff 1987]. The overriding effect of storage period may be a manifestation of after-ripening that protects the seed against precocious germination in appropriate growth conditions at wrong end of the growing season [Koller and Hadas 1982].

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