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Germination at Stress Temperatures and ABA Content of Pepper (*Capsicum annuum* L.) Seeds in Relation to Seed Development

Z. Sarıyıldız, I. Demir and N. Halloran
(Ankara University, Faculty of Agriculture, Department of Horticulture, Ankara, Turkey)

Summary

Changes in the percentage and time of germination at various temperatures (15, 25, 35 °C) and ABA (abscisic acid) content of pepper seeds harvested at 50, 60, 70 and 80 DAA (days after anthesis) were studied during growing seasons in 2001 and 2002. Seed dry mass ranged from 4.9 to 5.2 mg per seed in 2001, and from 4.7 to 5.0 mg per seed in 2002. However, seeds harvested at 70 DAA not only had maximum germination percentages, but also showed the lowest mean germination time for all three temperatures in both years. Delayed harvest (80 DAA) decreased germination percentages as a result of stress, and promoted viviparous germination in the mother plant. ABA content of the seeds increased gradually up to maturity, with values of 226 ng g^{-1} fresh weight (ng g^{-1} FW) at 70 DAA in 2001, and 203 ng g^{-1} FW at 60 DAA in 2002. It declined to a level between 85 and 90 ng g^{-1} FW in the final harvest of the both years. Germination at stressful temperatures and ABA contents of the pepper seeds with respect to seed development are discussed.

Zusammenfassung

Keimung bei Stresstemperaturen und ABA-Gehalt bei Paprika- (*Capsicum annuum* L.) Saatgut in Zusammenhang mit der Samenentwicklung. Während der Vegetationsperioden 2001 und 2002 wurden die Änderungen der Keimrate und der Keimdauer bei unterschiedlichen Temperaturen (15, 25, 35 °C) sowie der ABA- (Abscisinsäure) Gehalt von Paprikasaatgut, das 50, 60, 70 und 80 DAA (Tage nach der Anthese) geerntet worden war, untersucht. 2001 betrug die Samentrockenmasse 4.9–5.2 mg und 2002 4.7–5.0 mg pro Samen. In beiden Jahren wies das 70 DAA geerntete Saatgut bei allen drei Temperaturen die höchste Keimrate und die geringste durchschnittliche Keimdauer auf. Die spätere Ernte (80 DAA) reduzierte die Keimrate in Folge von Stress und förderte vivipare Keimung an der Mutterpflanze. Der ABA-Gehalt der Samen stieg bis zur Reife stufenweise an mit Werten, die 2001 226 ng g^{-1} Frischgewicht (ng g^{-1} FW) bei 70 DAA und 2002 203 ng g^{-1} FW bei 60 DAA betrugen. Zum Zeitpunkt der letzten Ernte sank er in beiden Jahren auf einen Wert von 85–90 ng g^{-1} FW ab. Die Keimung bei Stresstemperaturen und der ABA-Gehalt von Paprikasaatgut wird in Zusammenhang mit der Samenentwicklung wird diskutiert.

Key words. pepper – germination – abscisic acid – seed development

Introduction

Erratic and slow germination is a common phenomenon in pepper seeds grown under stressful conditions (Belletti and Quagliotti 1988). One feature expected of high quality seeds is to be able to germinate under adverse growing conditions. This has particular importance for crops such as pepper, in which seedlings are produced in the early spring at low temperatures for planting in the open-field, and in late summer at higher temperatures for glasshouse production in the Mediterranean region. Pepper seed quality is greatly influenced at the harvest stage as assessed by the rate and percentage of germination percentage and by storage longevity (Demir and Ellis 1992; Sanchez et al. 1993). While little information is available concerning the effect of the time of harvest on germination under stressful temperatures in peppers, seeds in tomato (a species in the same family as pepper) extracted from red/firm fruits 70 days after anthesis show maximum germination at low and high temperatures for earlier harvests compared to later harvests (Demir and Samit 2001).

Various physiological events occur during maturation of the seed on the mother plant. Proteins, lipids, and carbohydrates accumulate, resulting in an increase in seed dry weight. Ovule abscission marks the end of the accumulation phase, which is named **mass maturity** (maximum seed dry mass). One of the main biochemical changes in seeds occurs with the change in ABA content, which can be correlated with germination and vivipary (seed germination within the fruit) during development (Marrush et al. 1998; Still and Bradford 1998). In general, increases in ABA promote storage of reserved materials, and declines in ABA levels in late maturation stage have been associated with viviparous germination.

In this study we investigated pepper seeds for changes in the percentage and rate of germination at stressful temperatures, and changes in their ABA content with respect to developmental stage.

Table 1. Changes in seed moisture, dry mass and fresh seed germination in pepper seeds during development (DAA, days after anthesis) in growing seasons 2001 and 2002. The values represent means ± standard errors of the mean.

Days **A**fter **A**nthesis (DAA)	Growing season 2001 Seed moisture content (%)	2001 Seed dry mass (mg/seed)	2001 Fresh seed germination (%)	2002 Seed moisture content (%)	2002 Seed dry mass (mg/seed)	2002 Fresh seed germination. (%)
50	53 ± 2.0	4.9 ± 0.5	78 ± 3.0	61 ± 0.8	4.8 ± 0.6	70 ± 6.9
60	56 ± 0.4	5.1 ± 0.1	83 ± 1.0	55 ± 1.6	5.0 ± 0.1	85 ± 5.4
70	48 ± 0.0	5.2 ± 0.3	97 ± 1.7	60 ± 1.0	4.9 ± 0.3	96 ± 1.6
80	46 ± 1.4	5.0 ± 0.1	95 ± 1.7	53 ± 2.8	4.7 ± 0.2	97 ± 2.6

Materials and Methods

Pepper (*Capsicum annuum* L.) plants of the cultivar 'Sera Demre' were grown in open-field conditions in the growing seasons of 2001 and 2002. The maximum and minimum temperatures were recorded as 39 °C and 12 °C, respectively, in 2001, and 41 °C and 14 °C in 2002 throughout the growing season. The same plant cultivation practices were used as described in DEMIR (2002). Three hundred flowers during each year were tagged at full anthesis, and fruits were harvested at 50, 60, 70 and 80 days after anthesis (DAA) in both years. Seeds were extracted by hand, and 3 grams of fresh seeds in each harvest were stored immediately after harvest at –80 °C for ABA analyses. Fresh seed germination tests (radicle emergence) were carried out in three experiments of 50 seeds per experiment at 25 °C for 14 days. Seed moisture content (fresh weight basis) was determined according to ISTA rules (low temperature oven method, 103 °C, 17 h) (ANONYMOUS 1996). Remaining seeds were dried at 25 °C for 24 hours until seed moisture content is lower than 10 % and stored in sealed bags at 5 °C until used. Seed dry mass per harvest was calculated following the drying at 130 °C for 2 hours of three replicate batches of 50 seeds per batch; batches were then weighed after cooling in a desiccator containing silica gel. The mean dry mass of individual seeds was also derived. Germination at stressful temperatures and ABA content determinations were conducted within one month after completion of the final harvest in each growing season.

Germination tests were carried out on every harvest at temperatures of 15, 25 and 35 °C using three replicates of 50 seeds placed in between wetted paper towels (20 x 20 cm) for 14 days. Normal seedling percentages were recorded after every other day according to ISTA rules (ANONYMOUS 1996). Mean germination time was calculated according to the formula of ELLIS and ROBERTS (1980).

For ABA analysis 3 g of seed were freeze-dried, ground, and extracted with 80 % methanol overnight under constant shaking at 4 °C. The extract was processed according to the method described by LURIE and BEN YEHOSHUA (1986).

A Bio-Rad Model 2800 HPLC equipped with reversed-phase C_{18} column (Phenomenex) was used for ABA analysis. Samples (20 µL) of ethylated extract were analysed on column thermostated at room temperature, and peak heights used in ABA quantification.

Germination percentages were evaluated by ANOVA, following angular transformation and application of the least significant difference tests (LSD, P=0.05). Untransformed data were shown in the tables.

Results and Discussion

Seed moisture content decreased from 56 % to 46 % in growing season 2001 and from 61% to 53% in 2002. This was a typical trend in seeds of fleshy-fruited species in which seed moisture remains above 35 %, with seeds attached to the mother plant (WELBAUM and BRADFORD 1989; DEMIR and ELLIS 1992). Maximum dry mass (mass maturity) was already attained by 50 DAA (Table 1). Seed mass was the same among the harvests in both years and stabilised to about 5 mg/seed. However, fresh seed germination (radicle emergence) increased to 96–97 % at 70 DAA, and did not change subsequently, indicating that seed viability continued to improve after mass maturity. The interactive effect of temperature and harvest time on germination at different temperatures was significant in both years (P<0.05).

In 2001, the germination percentage of normally developed seedlings increased significantly by maturity, between 50 and 70 DAA, in which percentages were 89, 94 and 88% at 15, 25 and 35 °C, respectively. In 2002

Table 2. Normal germination percentages of pepper seeds at three different temperatures in relation to developmental stage (days after anthesis) in growing seasons 2001 and 2002. LSD (least significant difference, P<0.05) values are shown for the interaction between harvest stage and germination temperature.

Harvest (DAA)	Germination (%) 2001 15 °C	2001 25 °C	2001 35 °C	2002 15 °C	2002 25 °C	2002 35 °C
50	56	65	50	57	62	41
60	67	75	64	66	76	69
70	89	94	88	73	84	78
80	69	88	80	61	77	58
LSD (int.) P=0.05			6			4.5

Table 3. Changes in mean germination time in days (±SE) of pepper seeds at three different temperatures in relation to developmental stages (days after anthesis) in two consecutive years.

Harvest (DAA)	Mean germination time (days) 2001 15 °C		25 °C		35 °C		2002 15 °C		25 °C		35 °C	
50	12.0	±0.1	5.5	±0.1	6.4	±0.1	11.3	±0.0	4.7	±0.2	5.5	±0.0
60	11.3	±0.1	4.8	±0.2	5.5	±0.0	10.5	±0.2	3.6	±0.1	4.6	±0.2
70	11.0	±0.0	4.4	±0.0	5.3	±0.1	10.1	±0.2	3.1	±0.0	4.3	±0.0
80	12.5	±0.1	4.5	±0.1	6.0	±0.1	10.4	±0.1	3.7	±0.1	4.9	±0.1

percentages had decreased slightly at 25 °C, but greatly at 15 and 35 °C. A similar trend in germination percentages was also observed in the second year experiment. Overall germination percentages of seed lots in 2002 were lower than those in 2001. The lowest percentages were recorded in seeds at 50 DAA, being 57, 62 and 41 % for temperatures of 15, 25 and 35 °C, respectively, and the highest percentages at 70 DAA being 73, 84 and 78 % at those respective temperatures (Table 2). This finding was in agreement with the previous reports for various crops in which earlier seed harvests are more prone to germination under conditions of temperature, water, or salt stress (WELBAUM and BRADFORD 1991; DEMIR and SAMIT 2001; DEMIR et al. 2002).

The mean germination time values showed that the shortest time was observed at 25 °C compared to stressful temperatures. With the greater maturity of the seed, mean time to germination decreased. Seeds of 70 DAA had the minimum value at all three temperatures and for both growing years (Table 3). The mean germination time of pepper seeds is more severely affected at 15 °C than at 35 °C. The mean time of seeds of any harvest was found to be 10 or more days at 15 °C, while it varied between 4 and 6 days at 35 °C. One reason might be the fact that pepper is a warm season vegetable crop, and therefore low temperature stress can be more inhibiting on germination than high temperature. BELLETTI and QUAGLIOTTI (1988) also reported that low temperatures severely inhibit seedling growth and germination rate in pepper in early spring sowings.

Maximum seed quality determined as the highest germination percentages and shortest germination times at stressful temperatures in this study, varied among the lots in a statistically significant manner ($P<0.05$) and reached a maximum difference by 70 DAA. In contrast seed dry mass between lots remained the same. This finding supports the notion that seed quality continues to change after ovule abscission. GALAU et al. (1991) put forward the hypothesis that post-abscission improvements in quality requires some time attached to the mother plant until maximum seed quality is attained. Our results contrast with the hypothesis that maximum seed dry mass coincides with maximum seed quality in pepper, but are in agreement with those for a number of other crops (DEMIR and ELLIS 1992; SANHEWE and ELLIS 1996; STILL and BRADFORD 1998; DEMIR et al. 2002). It can be assumed that post-abscission seed quality improvement during development might be due to rapid loss of dormancy or an after-ripening effect.

ABA has been considered to be a major influence in maintaining and promoting seed development and preventing precocious germination in late maturation in various crops (SHRIVER and MUNDY 1990; BLACK 1991). BERRY and BEWLEY (1993) have indicated that ABA is also involved in the synthesis of storage proteins during development. ABA content of fresh pepper seeds during development was the lowest at the earliest time of harvest, with 52 and 16.8 ng g^{-1} FW for growing seasons 2001 and 2002, respectively, and ABA increased greatly by the maturity, attaining a maximum of 226 ng g^{-1} FW in seeds of 70 DAA in 2001, and 203 ng g^{-1} in seeds of 60 DAA in 2002 (Table 4). Subsequently, ABA decreased to 86.3 in 2001 and 93.0 ng g^{-1} FW in 2002 at the latest time of harvest for those seasons. Seed dry mass accumulation stopped by 50 DAA in pepper (Table 1). However, the ABA content of the seeds increased significantly up to 70 DAA in the first year, and up to 60 DAA in the second year, which shows that its increase was not dependent upon or affected by dry mass. This is in contrast with the results of BERRY and BEWLEY (1993) who studied tomato, and STILL and BRADFORD (1998) who studied rapeseeds in which they found that ABA content increased as seed dry mass accumulated. These findings could be due to starting the observation for changes in ABA and seed dry mass (protein) content at a much earlier stage of development, where our method was to begin observation at a later stage.

In the final harvests of both years, about 5–7 % of seeds showed radicle emergence in the fruit which coincided the time of decrease in ABA content. A number of studies have correlated vivipary with ABA levels in

Table 4. Changes in mean ABA contents (± SE) of fresh pepper seeds during development (days after anthesis) in growing seasons 2001 and 2002. Means associated with different letters in the same column are significantly different ($P<0.05$).

Harvest (DAA)	ABA content (ng g^{-1} fresh weight) 2001		2002	
50	52.0 ± 13.7	d	16.8 ± 2.4	d
60	127.4 ± 5.2	b	203.0 ± 8.0	a
70	226.0 ± 0.8	a	103.7 ± 6.5	b
80	86.3 ± 7.4	c	93.0 ± 3.3	c

the seeds in late maturation stages (GROOT and KARSSEN 1992; SMITH et al. 1993; MARRUSH et al. 1998). Abscisic acid-deficient mutant tomato seeds exhibited viviparous germination in overripe fruit. It was suggested that ABA inhibits precocious germination through preventing activation of germination enzymes, a mechanism shown in cotton (KRIZ et al. 1990) and pea (YOMO and VARNER 1973) seeds. However, BERRY and BEWLEY (1993) indicated that osmotic potential of sheath and locular tissue around the seeds are also as effective as ABA content in the prevention of vivipary in tomato.

Germination percentages and time in pepper seeds is under the inhibiting effect of low temperatures in early spring (open-field), and of high temperatures (protected cultivation) in summer sowings for transplant production. Our data indicate that a carefully controlled seed harvest time (70 DAA) would lower the inhibiting effect of temperature stress on pepper seedling production, and will help to promote the uniform germination of seedlings. Harvest of seed at a late time not only reduces seed germination at stressful temperatures, but also induces viviparious germination, which is associated with a low ABA content.

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Addresses of authors: Z. Sarıyıldız, I. Demir (corresponding author) and N. Halloran, Ankara University, Faculty of Agriculture, Department of Horticulture, Ankara, Turkey,
e-mail: demir@agri.ankara.edu.tr.