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Changes in germination and potential longevity of watermelon (*Citrullus lanatus*) seeds during development

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Abstract Changes in germination and potential seed longevity (K_i of the viability equation) were monitored during the development of watermelon (*Citrullus lanatus*) seeds grown in warm (minimum and maximum temperatures were 16.8 and 40.1°C) and cool (9.8 and 35.1°C) sites in 2002. Maximum seed weight (mass maturity, end of seed filling phase) was attained 25 days after anthesis (DAA) under both growing conditions. However, maximum germination was observed at 30 DAA in both sites. Potential seed longevity was achieved at 45 and 40 DAA, which were 20 and 15 days after mass maturity (the time of maximum seed dry weight) in warm and cool sites, respectively. When the maximum quality was achieved, fruit flesh was pale red, red, and 98–100% of the seeds were brown coloured. Seed dry mass and maximum potential longevity were consistently greater in cool than in warm growing sites. Dried seeds had higher germination percentages than those of fresh ones until mass maturity (25 DAA) at both sites.

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INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a warm season annual crop that originated in the dry areas of southern Africa. Watermelon requires a long growing season (4–5 months) and its yield is favoured by temperatures of c. 30°C (Hartman et al. 1988).

Optimum timing of harvest is a prerequisite for the production of high quality seed. The need for careful timing is especially important in warm season crops such as watermelon since elevated temperatures and high humidity may induce seed deterioration within the fruit (Dornbos 1995). Studies on seed maturation reveal considerable confusion regarding the timing of maximum seed quality (TeKrony & Egli 1997). Variability in the duration of the seed ripening phase with the production location and season in different crops has led to the development of various harvest indicators (Spurr et al. 2002). Mass maturity (time of maximum dry mass), formerly described as physiological maturity (Ellis & Pieta Filho 1992), was suggested as a good measure of maximum seed quality (germination and vigour) in a range of crops (Harrington 1972; TeKrony & Hunter 1995; TeKrony & Egli 1997). However, many recent reports cast doubt on the utility of maximum mass as an indicator of maximum seed quality (Welbaum & Bradford 1988; Demir & Ellis 1992a,b; Ellis et al. 1993; Zanakis et al. 1994; Demir & Samit 2001; Demir et al. 2002).

TeKrony & Egli (1997) proposed that these contradictory results may relate to differences in harvest timing or methods, drying techniques, and species. In species harvested as dry seed (maize, onion, soybean, etc.), maximum seed quality may be attained before or at mass maturity whereas in crops where seed develops in fleshy fruits (tomato, pepper), maximum seed quality occurs after mass maturity.

There has been no detailed examination of the development of seed quality attributes during maturation in watermelon. In subtropical regions that are potential seed production areas (i.e., southern Turkey) when high relative humidity is associated with high temperature, seed quality declines within a medium-range storage period (18–24 months). Determining the right harvest timing is a prerequisite to obtain high quality cucurbit seeds which, in turn, reduce seed quality loss during storage (Harrington 1959; Nerson & Paris 1988; Nerson 2002). This study examines changes in seed quality as indicated by germination percentages and potential seed longevity in serially harvested watermelon seed lots under warm and cool growing temperature regimes. The hypothesis that maximum germination and potential seed longevity are achieved at mass maturity was also tested.

MATERIALS AND METHODS

Plant husbandry and seed harvests

Plants of the watermelon cultivar 'Crimson sweet' were grown between March and October 2002 in: Hatay (south-eastern Turkey, near the Syrian border), a warm site; and in Canakkale (western Turkey, Aegean Sea coast), a cool site. Climatological values for warm and cool sites are given in Table 1. Seeds were sown in seedling trays on 3 and 18 March and seedlings were transplanted to the field on 4 and 17 May at the warm and cool sites, respectively. Plant spacing was 150 cm between and 100 cm within rows. Ammonium sulphate (15 kg/1000 m^2) and potassium nitrate (15 kg/1000 m^2) were applied 15 days after transplanting and again at the seed filling phase—10 days after anthesis (DAA). Furrow irrigation was applied weekly until mid June and then every 3 days until the end of the experiment. At each site, 60 flowers were tagged at full anthesis between 20 and 27 June and 5 and 12 July in warm and cool sites, respectively. Fruit harvests were arranged according to tagging day and were 15, 20, 25, 30, 35, 40, 45 DAA in both regions. Seeds were removed from the harvest fruit by hand. Seeds were washed in tap water and then dried on mesh trays in the dark for 2 days at 25°C at which time the seed moisture content was <10%. Seed moisture content was determined by the high-temperature oven method (130°C, 1 h) (ISTA 1996) before and after drying. Fruit flesh colour (pink, pale red, dark red, overmature) and seed colour (percentage basis in total number of seeds) were determined at each harvest. Following drying, seeds were stored at 5°C in glass jars until used. Seed dry weight determination was carried out by drying four replicates of 50 seeds per harvest at 130°C for 1 h and weighing after cooling in a desiccator with silica gel. Mean dry mass of individual seeds was then determined.

Germination test

The germination of fresh seed was compared to dried seeds. The seeds were germinated on moist rolled paper towels (10 × 10 cm, Filtrak, GmBH, Germany) at 25°C for 14 days (ISTA 1996). Each test involved four replicates of 50 seeds. Germination was considered to have occurred when the radicle was 2 mm long.

Determination of potential seed longevity (K_i of the viability equation)

Trials to determine the potential longevity of the various seed lots began on 8 November 2002, about a month after harvest. The seeds had been stored at 5°C at <10 % seed moisture content before this trial. The moisture content of each seed sample was adjusted to 16 ± 0.2% by humidifying seeds on top of

Table 1 Climatological summary daily maximum, minimum, and mean temperatures (°C) and monthly rainfall (mm) at cool and warm growing sites used for production of watermelon (*Citrullus lanatus*) seeds.

	Warm site				Cool site			
Month	Max. (°C)	Min. (°C)	Mean (°C)	Rainfall (mm)	Max. (°C)	Min. (°C)	Mean (°C)	Rainfall (mm)
May	33.2	16.8	21.1	11.6	29.4	9.8	18.2	0.1
Jun	37.4	21.3	25.5	9.0	34.1	12.6	23.3	0.1
Jul	39.2	25.4	28.2	0.1	35.1	19.2	26.6	0.8
Aug	40.1	25.0	27.8	15.2	35.0	17.2	25.7	0.1
Sep	37.2	21.7	25.6	27.5	29.6	10.3	21.4	1.3

mesh trays above water at 20°C and then holding them at 5°C for 7 days to allow moisture to equilibrate among them. For each lot (15 DAA was not taken because the very low germination), nine samples of 200 seeds were sealed in laminated aluminium foil bags. These were then stored in an incubator maintained at 40 ± 0.5°C. Samples were removed for germination tests at 3, 5, 7, 10, 14, 17, 21, 28, and 35 days. Germination tests were conducted as previously described on four replicates of 50 seeds.

Seed survival curves were fitted to the observed data by probit analyses using statistical package of SPSS (Statistical Program for Social Science) in accordance with the equation:

$$v = K_i - p/\sigma$$

where v is probit percentage viability after p days in storage, σ is the standard deviation of the frequency of distribution of seed deaths in time, and K_i is the seed lot constant (Ellis & Roberts 1980). Corresponding values of –3, –2, –1, 0, 1, 2, and 3 as probit are 99, 97, 85, 50, 15, 3, and 0.5 in percentages. Probit has been proposed as an appropriate test for seed longevity evaluations and comparison of the seed lots regarding storage potential (Finney 1962; Ellis & Roberts 1980).

Means of seed moisture and dry weight, fresh, and dry germination in each harvest were compared by Duncan's multiple range test by using SPSS. Angular transformation for percentages was carried out before analyses.

RESULTS

Mean temperatures during growing changed between 21.1 and 28.2°C at the warm site, and 18.2 and 26.6°C at the cool site, respectively. The cool site had almost no rainfall throughout the season but the warm site had reasonably high rainfall in August and September (Table 1). Maximum temperatures were above 35°C during the seed maturation period (July and August) at both sites.

Mass maturity, maximum seed dry weight, occurred at 25 DAA at both sites, averaging 33.4 mg/seed at the warm site and 43.0 mg/seed at the cool site. It remained stable subsequently ($P > 0.05$). At the time of mass maturity, fruit flesh was pink and between 43% and 50% of seeds were pale brown (Table 2). Fruit flesh became red by 35 DAA at the warm site but it took until 40 DAA at the cool site. Time of occurrence of maximum dry mass is the same after flowering but seeds produced in cool site were at least 10 mg/seed (20 DAA and onwards) heavier than those produced in the warm site throughout the development period (Table 3). Seed moisture content was 57.0% at the warm site and 53.2% at the cool site at mass maturity. Moisture content of the seed produced at the cool site was consistently lower than in the seed produced in the warm site. Seed moisture content stabilised after 25 DAA at 52–56% in the warm site and at 40–44% at the cool site. The moisture content of seed grown at the cool site was consistently lower than for seeds grown at the warm site. The difference was

Table 2 Changes in colour of fruit flesh and seed coat colour during development in watermelon (*Citrullus lanatus*) grown at cool and warm sites. (DAA, days after anthesis.)

	Warm site			Cool site		
DAA	Fruit flesh	%	Seed coat	Fruit flesh	%	Seed coat
15	White	80	White-yellow	White	100	Yellow
		20	Pale brown			
20	Pale pink	70	White-yellow	Pale pink	80	Yellow
		30	Pale brown		20	Pale brown
25	Pink	50	White-yellow	Pale pink	57	Yellow
		50	Pale brown		43	Pale brown
30	Pale red	87	Pale brown	Pink	97	Pale brown
		13	Yellow		3	Yellow
35	Red/firm	98	Brown	Red	98	Brown
40	Red/soft	98	Brown	Red/firm	98	Brown
45	Red/soft	100	Brown	Red/firm	100	Brown

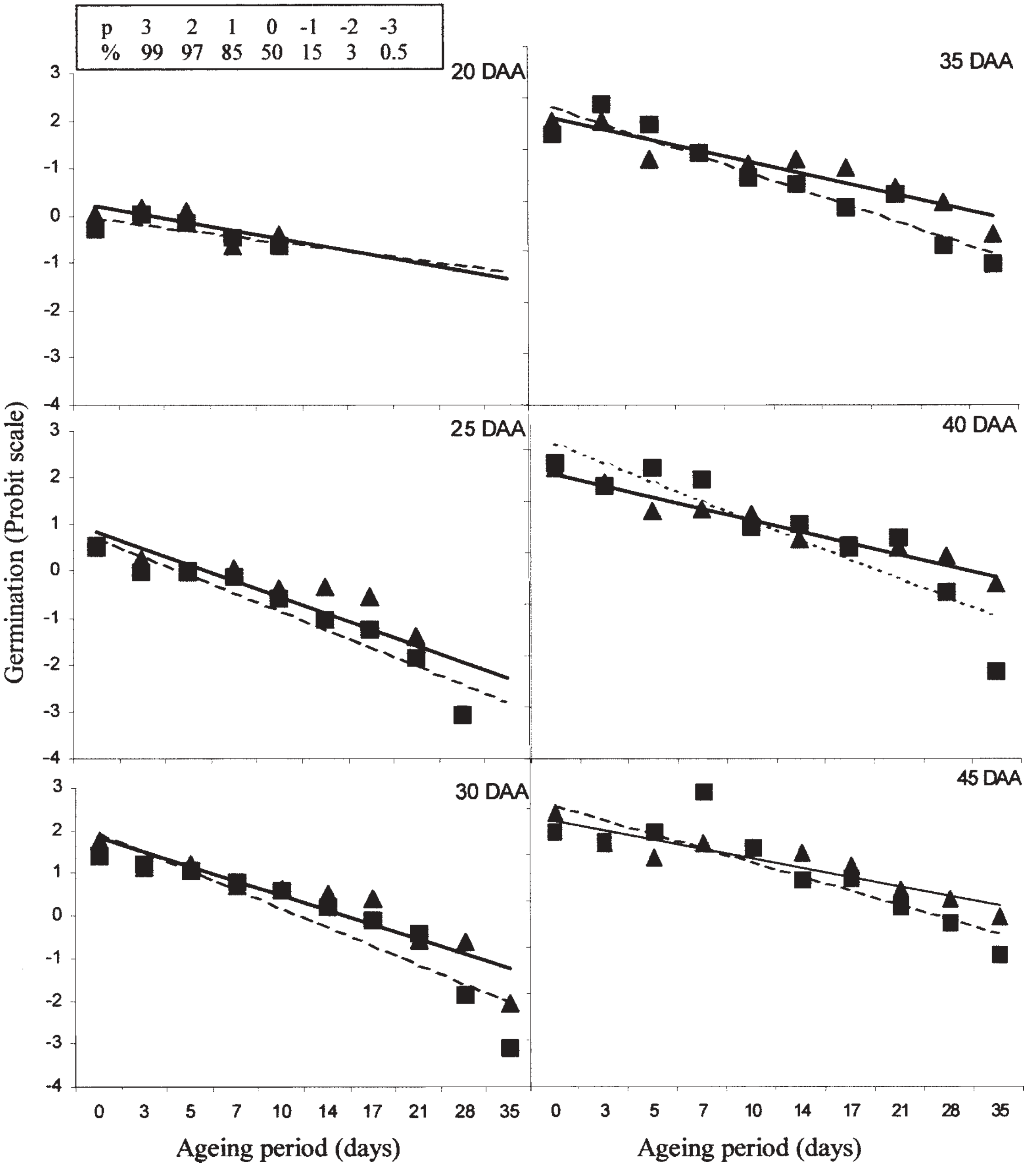

Fig. 1 Probit seed survival curves for watermelon (*Citrullus lanatus*) seeds harvested serially days after anthesis (DAA) during development from plants grown at cool (broken line, ■) and warm (solid line, ▲) sites. Corresponding probit (p) values in the percentages (%) are presented.

minimum at mass maturity (3.8%) and was greatest at the first harvest (14.3%) (Table 3).

Ability to germinate increased rapidly between the first and fourth harvests. In this period, the seed colour changed from yellow to pale brown and the fruit flesh colour went from white to pale red or pink. Germination percentages of dried seeds grown at warm site were higher than those of fresh ones in all

Table 3 Changes in seed dry weight (mg/seed), moisture content (%), fresh and dried seed germination percentages during development in watermelon (*Citrullus lanatus*) seeds grown at cool and warm sites. Upper and lower case letters are to compare means in the same line and column for each criterion, respectively ($P = 0.05$). (DAA, days after anthesis; NS, not significant.)

		Harvest (DAA)							
		15	20	25	30	35	40	45	Mean
Seed moisture (%)	Warm	79.3 a	69.5 b	57.0 c	56.5 c	52.5 c	53.2 c	57.8 c	60.8 A
	Cool	65.0 a	59.9 ab	53.2 b	43.7 c	40.1 c	42.5 c	45.0 c	49.9 B
Seed dry weight (mg/seed)	Warm	19.2 c	26.1 b	33.4 a	31.8 a	33.5 a	32.5 a	31.9 a	29.8 B
	Cool	20.8 c	33.9 b	43.0 a	43.1 a	43.8 a	43.3 a	42.4 a	38.6 A
Germination (fresh) %	Warm	0 e	13 d	34 c	80 b	79 b	82 b	96 a	55 B
	Cool	1 e	14 c	83 b	93 ab	97 a	99 a	100 a	70 A
Germination (dry) %	Warm	7 d	51 c	69 b	96 a	94 a	95 a	97 a	73 NS
	Cool	8 d	39 c	71 b	92 a	90 a	96 a	93 a	70 NS

Table 4 Changes in the potential seed longevity (K_i of the viability equation) of watermelon (*Citrullus lanatus*) seeds grown in cool and warm sites. Means with the same lower case letters in the same line and upper case letters in the same column are not significantly different ($P = 0.05$). (DAA, days after anthesis.)

		Harvest (DAA)					
		20	25	30	35	40	45
K_i	Warm	0.23 eA	0.54 dA	1.36 bB	1.41 bB	1.24 cB	1.55 aB
	Cool	–0.67 eB	0.51 dA	1.60 bA	1.51 cA	1.81 aA	1.81 aA

seed lots except seeds of 45 DAA. This was also the case for seed lots of cool production site until mass maturity.

Figure 1 shows the probit seed survival curves under accelerated ageing conditions based on transformed values from germination percentages of samples taken serially from ageing environment until 35 days. The longevity of seed indicated by intercept value (K_i) under adverse storage conditions increased as the seed matured until 45 and 40 DAA at the warm and cool sites, respectively. The higher estimates of K_i showed that potential longevity continued to increase subsequent to mass maturity at both sites (Table 4). The greatest potential of longevity (1.55) at the warm site ($P < 0.05$) was shown by seeds harvested 45 DAA which is 20 days after mass maturity. At the cooler site, maximum potential longevity was attained 40 DAA as 1.81 or 15 days after mass maturity. Seeds grown at cool site had higher potential longevity than that of warm during development stage except for the first two harvests.

DISCUSSION

It has been reported that the quality of watermelon and cucurbit seeds depends on the seed maturation stage at harvest (Nerson & Paris 1988; Nerson 2002). Nerson (2002) reported that fully matured watermelon seeds (35–49 DAA) not only had higher germination percentage and rate but also retained viability over 10 years at 10°C/45% relative humidity. However, immature (21 DAA) and half-mature (28 DAA) seeds started to lose germinability after 4 and 6 years, respectively. In agreement with this finding, more mature seed lots (40–45 DAA) also had longer seed longevity (higher K_i values) than those of half-mature and immature lots in this work.

K_i, the seed lot constant of the viability equation, has been proposed as a good indicator of seed quality and vigour in many crops (Ellis & Roberts 1980). The results presented here, for the changes during development in the potential longevity of watermelon seeds produced in differing environments, are in agreement with the results of earlier studies of other crops (Demir & Ellis 1992a,b; Zanakis et al. 1994; Demir & Samit 2001; Demir et al. 2002), that maximum seed longevity was attained some time (5–20 days) after mass maturity. These results contradict the widely accepted hypothesis that maximum seed quality is attained at the end of the seed filling phase and that viability and vigour then decline (Harrington 1972).

TeKrony & Egli (1997) proposed that in crop species harvested as dry seeds, maximum seed quality is attained before or when maximum dry mass is achieved, but in plants where the seed is contained within a fleshy fruit maximum seed quality occurs some time after occurrence of maximum dry mass. Our results with fleshy fruited watermelon are in agreement with their findings.

High temperatures at the warm site reduced potential longevity compared with seeds grown at the cool site (Table 4). Moreover, watermelon seeds generated at the warm site had lower dry mass than those generated at the cool site. Yet, the time from anthesis to maximum seed dry mass was the same at both sites (Table 3). High temperatures, then influenced seed yield by reducing dry mass per seed. Fresh seed germination percentages were higher at the cool site than at the warm site throughout development. It is likely that the combination of higher seed moisture content, growing temperatures, and rainfall at the warm site compared to the cool site may have inhibited fresh seed germination. High production temperatures were reported to be deleterious to seed yield and quality, particularly when combined with high seed moisture content and rainfall during maturation (TeKrony et al. 1980; Ellis et al. 1993).

Seed maturation was associated with changes in fruit flesh and seed coat colour during development (Table 2). When maximum seed quality was obtained the fruit flesh was red and 98–100% of the seeds were brown. Colour of seed and fruit flesh can be used as harvest indicators in watermelon. Fruit colour, umbel dehiscence, and seed moisture content have been used as harvest criteria in various vegetable crops (Edwards & Sundstrom 1987; Gray & Ward 1987).

At both growing sites, dried seeds had higher germination percentages than fresh seeds until mass maturity. The beneficial effects of drying continued in seeds grown at the warmer site until the final harvest, but not in those grown at the cool site (Table 3). Present results indicate that high temperatures during seed maturation on the mother plant may likely induce seed germination following drying. Demir & Yanmaz (1998) reported that the drying (<10%) cucumber seeds improved germination compared with fresh seeds. Similarly, the enhancing effect of drying on the germination of watermelon seeds was also indicated by Nerson (2002). The underlying mechanism of the heat-induced germination enhancement of watermelon seeds needs to be resolved.

The increased seed weight achieved in the cool environment was associated with increased seed longevity, particularly subsequent to mass maturity. The relationship between seed weight and seed quality is unclear. Spurr et al. (2002) indicated that there is a positive relationship between seed weight and germination rate and uniformity in onion. However, Ocena Gil et al. (1991) found no effect of seed weight on seed quality in the same species. The results of this study support the positive relationship between seed weight and seed quality assessed by potential longevity (Tables 3 and 4). Seed longevity of seeds grown in the cool environment had longer longevity than those grown in the warm one ($P < 0.05$) between 30 and 45 DAA (Table 4). This may be because of the larger amount of reserve material in heavier seeds which was reported to be one component in extended seed longevity (Priestley 1986). Although we have not measured seed yield, obtaining heavier seeds might result in higher seed yield at the cool site. Higher temperatures might cause reduced pollen viability and pollination rate and in turn a reduced number of seeds per fruit. Moreover, seeds have insufficient biomass accumulation (small seeds) in high temperatures (George 1985).

Viviparous germination (preharvest seed germination within the fruit) causes quality loss in the fleshy fruited vegetables in late maturation stages. Delaying harvests of tomato and pepper seeds until 80 DAA and later resulted in 2–5% of viviparous germination (Demir & Ellis 1992a,b). In this work, vivipary was not observed in watermelon seeds even in the latest harvest, even though seeds have a very high moisture content throughout the developmental stages. In agreement with our conclusions, Welbaum & Bradford (1988) reported that high seed abscisic acid content, low osmotic potential of fruit flesh, and the presence of a protecting cap around the endosperm and embryo might play a role to prevent viviparous germination of cucurbit seeds.

It can be concluded that watermelon seeds with maximum germination and storage should be harvested when the fruit is 40–45 days old when grown at either warm or cool sites. This was 15–20 days after occurrence of mass maturity of the seed and coincided with the time of red and firm fruit flesh and 98–100% of seeds were brown coloured.

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