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Large Sunflower Seeds are Characterized by Low Embryo Vigor

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ABSTRACT. **The cause for the differences in germination ability of large and small confection sunflower (*Helianthus annuus* L.) seeds was investigated over 3 years. The source–sink relationship was manipulated to better explore the differences between seeds of various sizes and to study the role of the embryo and the pericarp (hull) in controlling germination ability. Percent germination of large seeds was significantly lower than that of small seeds when tests were performed at 15 °C. Increasing the ratio of leaf area to number of developing seeds caused an increase in mean seed mass, but resulted in a lower percentage of germination. Seed vigor, as measured by mean time to germination or to emergence of hulled seeds or by rate of root elongation, was negatively correlated with embryo mass, indicating that the low vigor of large seeds is not due to the mechanical barrier imposed by the hull. Analysis of electrolyte leakage confirmed the hypothesis that the low quality of large seeds results from a disturbance during the process of seed development.**

Rapid germination is essential for seeds to overcome stresses such as water or oxygen deficits, salinity, soil crusting, pathogens and insects (Hegarty, 1978). A seed's ability to emerge early is thought to be a function of the viability and vigor of the seed lot. Factors known to influence seed vigor include genotype, the nutrition and growth conditions of the mother plant, the physiological maturity of the seed at harvest, the physical handling of the seed during processing, seed moisture content and temperature during storage (Perry, 1981).

As early as 1893, Boss examined the effect of seed size on the subsequent growth and ultimate yield of plants. In many investigations since then, a positive correlation has been found between seed size and vigor (Halmer and Bewley, 1984). A higher value of thousand-seed mass was associated with a shorter time to emergence (Maranville and Clegg, 1977). Seed size is affected by changes in resource supply (Sackston, 1959; Siddiqui and Brown, 1977) and therefore a higher seed mass usually indicates a higher level of reserves, resulting in rapid germination and emergence. However, Naylor (1981) reported that seed lots of Italian ryegrass, characterized by higher seed mass, did not germinate as well as lots of smaller seeds.

The sunflower (*Helianthus annuus* L.) propagule is botanically an achene (but is considered to be a seed in this paper), which consists of a kernel and adhering pericarp (hull). Large seeds usually have thick hulls and are not well filled, whereas small seeds usually have thin hulls (Knowles, 1978). Confection sunflowers are large-seeded cultivars, suited to consumer demand for seed quality, i.e., seed of a specific size, shape, easily removable hull, and suitable roasting characteristics. These cultivars are characterized by a low percentage of germination and poor vigor. The mechanical restraint against radicle elongation imposed by the thicker hulls of big seeds may inhibit germination and could explain the differences in quality among confection sunflower seeds of various sizes. However, the differences in percent germination may also be due to low vigor of the embryos that developed in big seeds.

The aim of this research was to determine the role played by the embryo and hull in controlling germination ability of confection sunflower seeds.

Materials and Methods

FIELD TRIALS. Three field experiments were conducted in commercial sunflower ('DY-3') plots near Rehovot, in the center of Israel. In 1993, ≈150 plants were randomly selected 28 d after emergence and divided into two predicted head sizes according to leaf area (preliminary observations showed a direct relationship between leaf size and head diameter), and plants of the two groups were arranged into five blocks. An additional effect on seed size was obtained by manipulating the source–sink relationship. The treatments consisted of 1) control, 2) removal of every other leaf at the end of the flowering period, and 3) removal of achenes from the peripheral zone of the head (≈33% of the head diameter) at the end of the flowering period.

In 1994, head size was manipulated via plant density: a high density (conventional agrotechnique) of 3.2 plants/m^2 yielded small heads, and a low density of 1.6 plants/m^2 yielded large ones. Source–sink relationship manipulations were similar to those of 1993, with an additional treatment consisting of early leaf removal (removal of every other leaf just after its appearance).

In 1996, seed size was manipulated by plant density only: the high density consisted 5.2 plants/m^2 and the low density of 1.6 plants/m^2.

Heads were harvested at the end of the season and achenes were collected from a strip in the middle of each head (between its center and periphery). The width of this strip constituted ≈33% of the head diameter.

SEED GERMINATION. Before each germination test, achenes were surface-sterilized with aerated 2.5% NaOCl for 10 min (6 min for hulled seeds) and then rinsed with running water for 5 min. Six replications of 10 achenes each were sown in 9-cm petri dishes on a layer of cotton wool and moistened with 20 mL of distilled water (germination test of hulled seeds was conducted on three layers of Whatman no. 2 filter paper moistened with 8 mL of distilled water). Germination took place in the dark at either 15 ± 0.5 or 25 ± 1 °C (adjusted incubator), and was defined as the stage at which

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Table 1. Effect of plant density and source–sink manipulations of sunflower plants on the mass of various seed components.

Year	Head size	Achenes	Seed mass (mg) Hulled seed	Pericarp	Hulled seed/ achene ratio
1993	Large				
Control		161 a[z]	82 a	79 a	0.51
Leaf removal		154	79	75	0.51
Seed removal		214	110	104	0.51
	Small				
Control		114 b	60 b	54 b	0.53
Leaf removal		119	62	57	0.52
Seed removal		181	95	86	0.52
1994	Large				
Control		252 a	111 a	141 a	0.44
Early leaf removal		212	102	110	0.48
Late leaf removal		240	118	122	0.49
Seed removal		259	113	146	0.44
	Small				
Control		229 a	106 a	123 a	0.49
Early leaf removal		196	99	97	0.51
Late leaf removal		208	103	105	0.50
Seed removal		245	119	126	0.49
1996	Large	247 a	111 a	136 a	0.45
	Small	183 b	86 b	97 b	0.47

[z] Mean separation between head sizes (controls only) in each year by Duncan's multiple range test at $P = 0.05$.

the seed radicle had reached 5 mm in length. Evaluation of germination ability was based on the percentage of germinated seeds or the mean time (in days) to germination (MTG) where MTG = (no. of days to germination × No. of seeds germinated/ Total no. of seeds germinated

Radicle length was measured on the date of germination (protrusion of each seed coat) and after 4 d of radicle elongation. Data present root elongation during those 4 d.

The emergence test included six replications of 20 seeds that were sown in sterilized sand at a 1.5 cm depth. Emergence took place in an incubator adjusted to a constant temperature of 25 °C with a 14-h photoperiod.

Conductivity test. In 1993, the test design consisted of randomized complete blocks with three replicates of 10 seeds each. The hulled achenes were soaked individually for 4 h in 3.5 mL deionized water. Conductivity of the seed leachate was measured in (amps per seed with a seed analyzer (ASA 610; Agro Science Inc., Michigan). The seeds were carefully removed and placed on three layers of Whatman no. 2 paper (moistened with 8 ml of distilled water) in petri dishes. The dishes were placed in an incubator at 15 °C for 4 d and percent germination was measured. In 1996, the test included six replications of eight seeds each. Conductivity of a single seed's leachate (in 1 mL deionized water) was measured using a conductivity meter (model CDM 83; Radiometer, Copenhagen).

Results

A positive relationship between head size and mean seed mass was established in all 3 years (Table 1). Mean seed mass of control plants having small heads was ≈70% of that of control large heads. In 1994, we tried to impose differences in head size by altering plant density. Reducing plant density from 3.2 plants/m^2 (commercial stand) to 1.6 plants per m^2 resulted in larger heads (diameter of 25.2 cm vs. 19.4 cm, respectively) and higher seed mass. However, the effect of density on seed size was less pronounced than the differences observed in 1993, and mean seed mass of high-density heads was ≈90% of that on low-density heads. The effect of plant density on mean seed mass was also observed in 1996, when mean seed mass of small heads (high density) was ≈74% of that of large heads (low density). Removal of leaves at the end of the flowering period had no effect on the mass of the various seed components. However, seed removal resulted in a significantly higher mass of the remaining seeds (Table 1). Again, the effect of source–sink manipulation on seed mass was not significant in the 1994 experiment.

It is interesting to note that the hulled seed to achene ratio was similar in all treatments for all 3 years (Table 1).

Percent germination was higher in small seeds than in big ones (Table 2). This effect was pronounced during the first month after harvest and was almost eliminated after 18 weeks. It is important to note that percentage of germination was relatively low for all

Table 2. Percent germination and mean time to germination (MTG) of large and small seeds. Germination tests were performed at 25 °C, 1, 4, 18, and 47 weeks from harvest date (31 July 1996).

Seed size	Large	Small
1 week		
Germination (%)	28 a	55 a
MTG (days)	5.4 a	3.6 a
4 weeks		
Germination (%)	23 b[z]	52 a
MTG (days)	4.2 a	2.8 b
18 weeks		
Germination (%)	64 a	68 a
MTG (days)	3.3 a	2.7 a
47 weeks		
Germination (%)	34 b	61 a
MTG (days)	3.3 a	2.4 b

[z] Mean separation between columns by Duncan's multiple range test at $P = 0.05$.

Table 3. Percent germination and mean time to germination (MTG) of large and small seeds. Germination tests were performed 18 weeks after harvest (31 July 1996) on either cracked or hulled seeds at 25 or 15 °C.

Seed size	Large	Small
Cracked (25 °C)		
Germination (%)	92 a	99 a
MTG (days)	2.6 a^z	2.1 b
Hulled (25 °C)		
Germination (%)	100 a	100 a
MTG (days)	2.2 a	2.0 b
Hulled (15 °C)		
Germination (%)	100 a	100 a
MTG (days)	4.1 a	3.3 b

^{z}Mean separation between columns by Duncan's multiple range test at $P = 0.05$.

seeds, even after 18 weeks. MTG was lower for small seeds on all dates, however the differences were only statistically significant 4 weeks after harvest (Table 2). The thicker and heavier pericarp of the big seeds may explain the slower rate of germination (higher MTG) relative to small seeds. To explore the role of the pericarp in controlling germination ability of sunflower seeds, germination tests were performed on either cracked (small crack at the radicle end of the pericarp) or hulled seeds. Small cracks in the pericarp resulted in 92% and 99% germination for big and small seeds, respectively, whereas removal of the pericarp enabled 100% germination of all seeds (Table 3). Although no differences were observed in percent germination, MTG was significantly longer in big seeds. These differences were more pronounced at 15 °C than at 25 °C (Table 3). Positive correlations between hulled-seed size and MTG were also found in the 1993 and 1994 experiments (Fig. 1), with r^2 values of 0.85 and 0.67 for 1993 and 1994, respectively, statistically significant for both years.

Percent germination is a measure of seed viability only, whereas MTG provides a measure of seed quality or embryo vigor. To further explore whether the slower germination of big seeds was due to the larger pericarp or to low embryo vigor, hulled seeds were subjected to an emergence test at 25 °C. Percent emergence of hulled seeds after 14 d was 74 for small seeds as compared to 48 for large seeds with $P = 0.059$ (Table 4). Mean time to emergence (MTE) for big seeds was 8.1 d, which was significantly longer than the MTE for small seeds (6.6 d).

The MTG and MTE differences between large and small hulled seeds indicated that the pericarp is not the only reason for the low vigor of large seeds. To further characterize the vigor of various seed sizes, we determined the elongation rate of the radicle at 15 °C. This rate was 6.0 mm·d^{-1} for small seeds, as compared to 4.1 for large seeds. These differences were not statistically significant ($P = 0.052$). However, significant negative correlations between seed mass and radicle elongation during the first 4 d after germination were found for seeds harvested in 1993 and 1994 (Fig. 2). The r^2 values of 0.54 and 0.30 in 1993 and 1994, respectively, are significant at $P = 0.01$ with the respective degrees of freedom.

In 1996, mean electrolyte leakage from large seeds was 17.9 µA·h^{-1} compared to 9.9 in small seeds (Table 5). It can be argued that a comparison on the basis of a single seed is misleading because the membrane area of the large seeds is higher than that of small seeds. This test should, therefore, not reflect true differences in membrane function. However, analysis of electrolyte leakage per unit seed mass also indicated significantly higher leakage from large vs. small seeds. Moreover, a measurement of electrolyte leakage from 180 seeds from the various treatments (1993 experiment) indicated a positive correlation between mean seed size and the mean conductivity for each treatment (Fig. 3A). Again, a positive (though not statistically significant) correlation was found between seed mass and conductivity per unit seed mass (Fig. 3B). When analyzed for the 1996 experiment, the correlation coefficient between seed size and electroconductivity per seed mass unit was 0.78, which was statistically significant ($P = 0.01$).

Discussion

Germination of confection sunflower seeds is often difficult, resulting in poor field emergence. The problem of poor emergence under field conditions is even more pronounced in confection varieties characterized by large seeds and a thick pericarp. A negative relationship between germination ability and seed size was also found in our studies (Table 2). These differences were more pronounced at low temperature (15 °C). For example, in 1993, germination percentages of seeds with average masses of 114 and 119 mg were 69 and 61, respectively, whereas the germination percentages of seeds with average masses of 181 and 214 mg were 26 and 20, respectively. It should be remembered that under field conditions, soil temperature at the time of sowing is closer to 15 than to 25 °C, and the germination tests at the lower temperature may therefore better reflect the status under field conditions.

The relationships between seed size and vigor are usually explained by the amount of food reserves or the efficiency of metabolism (Halmer and Bewley, 1984). In our study, the amount of reserves was altered by manipulating plant density and source–sink relationships. It is interesting to note that leaf removal had only a limited effect on seed mass, even when every other leaf was removed just after its appearance (Table 1). The most interesting results obtained in this set of experiments pertain to the effect of source–sink manipula-

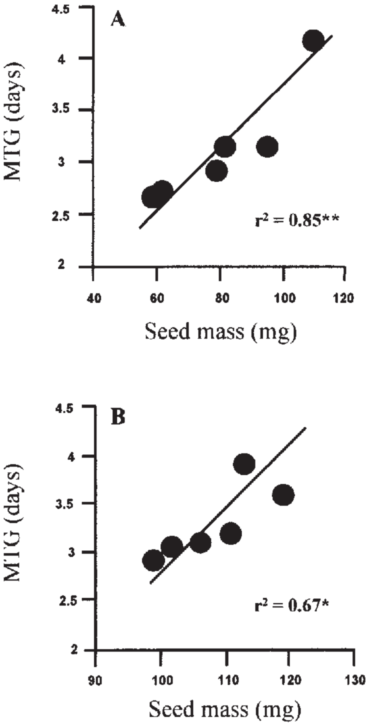

Fig. 1. Relationship between hulled-seed mass and mean time to germination (MTG) after 14 d at 15 °C. Data obtained from seeds developed in 1993 (**A**) and 1994 (**B**). Linear regressions and correlation coefficients were calculated for the respective data. *,**Significant at $P \leq 0.05$ or 0.01, respectively.

Table 4. Percent emergence and mean time to emergence (MTE) of large and small seeds. Emergence tests were performed 15 weeks after harvest (31 July 1996) on hulled seeds at 25 °C.

Seed size	Large	Small
Emergence (%)	48	74
MTE (days)	8.1 a^z	6.6 b

^{z}Mean separation between columns by Duncan's multiple range test at $P = 0.05$.

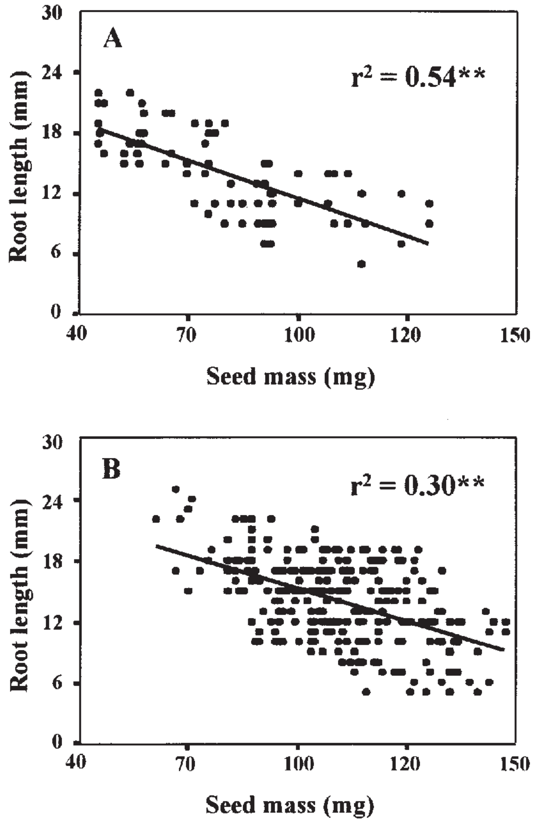

Fig. 2. Relationship between hulled-seed mass and root length after 4 d from radicle protrusion of each seed coat at 15 °C. Data obtained from seeds developed in 1993 (**A**) and 1994 (**B**). Linear regressions and correlation coefficients were calculated for the respective data. **Significant at $P \leq 0.01$.

Table 5. Electroconductivity of large and small seeds developed under two different plant densities in 1996. Conductivity tests were performed on hulled seeds and values are expressed per seed and per unit seed mass in $\mu A \cdot h^{-1}$).

Seed size	Large	Small
Conductivity/seed ($\mu A \cdot h^{-1}$)	17.9 a^z	9.9 b
Conductivity/g seed ($\mu A \cdot h^{-1} \cdot g^{-1}$)	151 a	105 b

^{z}Mean separation between columns by Duncan's multiple range test at $P = 0.05$.

tions on seed quality. In contrast to the general concept, increasing the ratio of leaf area per number of developing seeds did not increase seed vigor. On the contrary, a significant positive correlation was found between seed mass and MTG (Fig. 1), which is often used as a measure of seed vigor.

A prerequisite for germination is the ability of the embryo to weaken the mechanical restraint opposing the radicle tip. If seed size is positively correlated with the thickness of the seed coat, germination rate of big seeds is expected to be slower (Maranville and Clegg, 1977). An early report indicated that smaller sunflower seeds have relatively bigger kernels with thicker walls as compared to big seeds (Lofgren, 1978). However in a study of other varieties under different conditions, the hull to seed ratio (on a mass basis), was found to be smaller for small seeds than for big ones (Zimmerman and Zimmer, 1978). In addition to mechanically restraining the growth of the embryo, the seed coat has a marked influence on the ability of many seeds to germinate by regulating water uptake, gaseous exchange, and diffusion of endogenous germination inhibitors (Ikuma and Thimann, 1963, Werker, 1981). Hull removal was therefore expected to reveal the advantage of big seeds in terms of food reserves. However, analyses of emergence and root elongation rate, which provide other measures of seed vigor, again indicated a negative correlation between hulled seed mass and seed quality (Table 4 and Fig. 2). These results further support the hypothesis that the low germination percentage of big seeds is not only due to the mechanical barrier imposed by the seed coat, but to low embryo vigor. As indicated, seed coats (or pericarp in the case of sunflower) inhibit gas exchange. Therefore, it may very well be that a thick pericarp, as in the case of large seeds, limits the oxygen supply during embryo development or inhibits the release of endogenous inhibitors, resulting in impaired embryo development and low vigor.

Membrane function is one of the key factors controlling seed quality. Abdul-Baki (1980) suggested that seed deterioration is predominantly due to disorganization of the organelle–membrane system. Analysis of different sunflower seed lots revealed that lipid concentration in small seeds (with a high percentage of germination) was higher than in big seeds (which were poor germinators), whereas protein content was similar for all seeds (Reuzeau et al., 1992). Interestingly, a significant negative correlation has been found between oil concentration and sunflower-seed size (Lofgren, 1978). These results can be interpreted on the basis of food reserves, because triglycerides constitute the major reserve component in those seeds. However, they can also be explained on the basis of membrane integrity. As indicated earlier, based on the source–sink manipulations in our study, differences in reserve level could not explain the poor quality of the large seeds. Furthermore, electrolyte leakage was significantly higher in

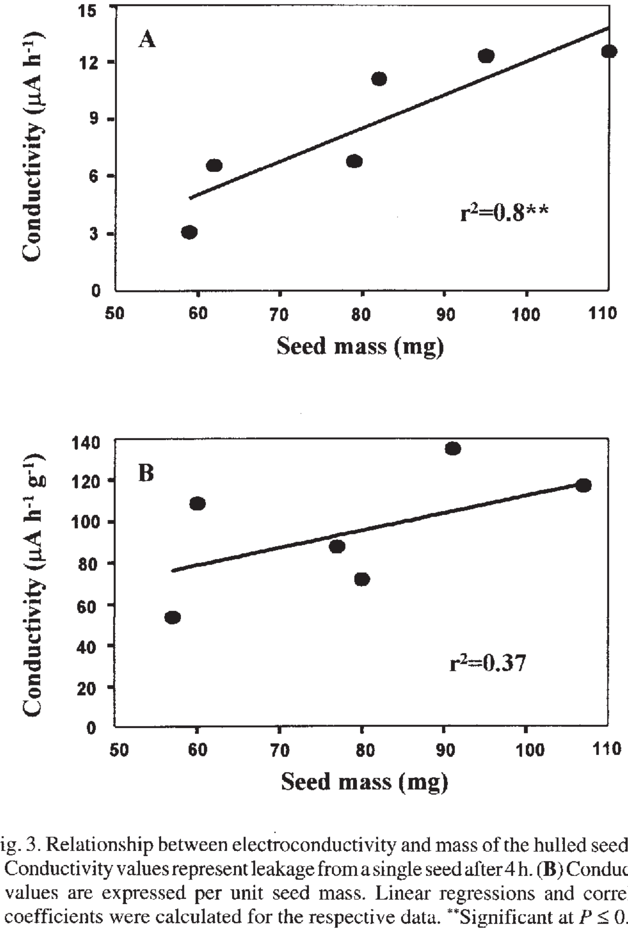

Fig. 3. Relationship between electroconductivity and mass of the hulled seeds. (**A**) Conductivity values represent leakage from a single seed after 4 h. (**B**) Conductivity values are expressed per unit seed mass. Linear regressions and correlation coefficients were calculated for the respective data. **Significant at $P \leq 0.01$.

large seeds vs. small seeds, even when expressed on a unit mass basis (Table 5). The positive correlation between seed size and electrolyte conductivity was confirmed in experiments performed in two different years, indicating that membrane function in big seeds is impaired, resulting in a higher rate of electrolyte leakage.

Several studies have indicated that the inability to germinate arises from embryo dormancy and seedcoat-imposed dormancy (Corbineau et al., 1988, 1990; Le Page-Degivry and Garello, 1992). These dormancies are gradually eliminated during dry storage of the seeds (Corbineau et al., 1990; Cseresnys, 1979). It may very well be that dormancy of large seeds differs from that of small ones. One can argue that residual dormancy persists even after all seeds are germinable, influencing the rate of germination. In this case, germination rate continues to increase after different periods of storage, although all seeds are able to germinate (Bradford, 1996). The increase in percent germination with a concomitant decrease in MTG (Table 2) concurs with earlier publications related to seed dormancy. However, the fact that MTG values remained constant after 18 weeks of storage (Table 2) suggests that, at this time point, no residual dormancy persisted in both small and large seeds. It is therefore logical to assume that the higher values of MTG for large seeds, 18 weeks after harvest, reflect lower vigor. Moreover, when the pericarp was removed from seeds stored for 18 weeks, they all germinated 100% at 15 and 25 °C (Table 3). Nevertheless, significant differences were found in MTG values between small and large seeds. These results further support the conclusion that large seeds are characterized by low vigor.

The fact that small seeds reach their maximal germination rate after a shorter period of storage (Table 2) indicates that large seeds have deeper dormancy. A characterization of dormancy in seeds of various sizes and the role of dormancy in inhibiting field emergence of large seeds are currently under investigation. Nevertheless, the accumulated data obtained from several independent analyses, such as MTG, emergence, root elongation rate and electrolyte leakage, suggest that the poor germination of large sunflower seeds is also due to low embryo vigor. Because seeds were harvested by hand, the low quality of large seeds can not be attributed to mechanical damage. It is therefore logical to assume that this quality results from a disturbance during their development. The challenge lying before us now is to define the cause of this disturbance during seed growth and maturation.

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