Seed Germination Performance and Mucilage Production of Sweet Basil (*Ocimum basilicum* L.)

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ABSTACT

Sweet basil (*Ocimum basilicum* L.) is a warm season herb usually propagated from seeds. Establishment of basil is difficult as seed germination may be limited, particularly during field seeding at cold soil temperatures. The germination of six cultivars ('Italian Large Leaf', 'Italian Large Leaf' 35X, 'Nufar', 'Genovese', 'Genovese Compact Improved' and 'Aroma 2') of sweet basil seeds were tested on a one dimensional thermo-gradient table over temperatures ranging from 0 to 50°C. At temperatures below 20°C, germination among cultivars was more variable and the mean time to germination (MTG) increased to greater than 25 days for some cultivars. Germination declined sharply and had a sudden termination at high temperatures above 40°C for all six cultivars. There were statistical differences among the cultivar base temperatures, which ranged between 10.1 and 13.3°C. The optimal and ceiling temperatures for germination were similar and did not differ statistically among the cultivars compared in this study. The average optimal temperature for all cultivars was 35 ± 0°C, while the average ceiling temperature was 43 ± 1.3°C. Stored seeds (> 5 years) had lower seed vigor and lower germination percentage, also lower ceiling temperature compared with the fresh seeds of the same cultivar ('Italian Large Leaf'), but the base temperatures were the same for both new and old seeds.

Sweet basil (*Ocimum basilicum* L.) seeds produce a thick layer of mucilage around the pericarp within minutes after hydration. Mucilage is most prevalent among plant species adapted to surviving in arid sandy soils, though its significance in determining ecological fitness is unclear. The mucilage produced by seeds is reported to be composed of cell-wall polysaccharides that are deposited in testa pericarp cells during development. In this study, sweet basil seeds were examined using light and environmental scanning electron microscopy. The mucilage of basil seeds is held together by columnar structures that unfolded from the pericarp and helped hold and stabilize the mucilage to the outer surface. The mucilage was removed using diluted hydrochloric acid to compare performance of seeds with and without mucilage. Mucilage removal did not inhibit seed germination under ideal laboratory conditions but decreased the

water content of seeds significantly. The water content of intact seeds was almost 4 times greater than seeds without mucilage. Mucilage enabled seeds cling to an incline board set to a steeper angle than seeds without mucilage. The fully hydrated seeds approached zero water potential, so the mucilage did not prevent seeds from fully hydrating. Soil (media) germination testing showed the seeds with mucilage had higher germination percentage than the seed without mucilage on several different types of media. Seeds with mucilage also had higher survival percentages after 10 days on different types of media. Basil seeds mucilage acts as a reservoir to hold loosely bound water at high water potential so it is available for seed germination and early seedling development.

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Chapter 1

Literature Review of Germination Performance and Mucilage Production by Sweet Basil (*Ocimum basilicum* L.) Seeds

Sweet basil (*Ocimum basilicum* L.) is a frost sensitive low-growing herb which belongs to the family Lamiaceae (mint family). Basil is native to southern Asia and when grown in North American is an annual summer crop. Basil has ovate pointed leaves that are simple with pinnate venation, 20-60 cm length and white-purple flowers that are normally self-pollinated (Fig. 1). There are more than 60 cultivars which differ in appearance and taste (Putievsky, 1983). Sweet basil is cultivated over a temperature range between 7 and 27°C, requires 0.6 to 4.2 m annual precipitation and a soil pH ranging from 4.3 to 8.2. It is susceptible to frost and chilling injury, and develops best under long days, in full sun, and well-drained soils (Simon, 1998). Most sweet basil cultivars take 68 to 78 days from seeding to vegetative harvest. Sweet basil is cultivated for the fresh market as a culinary herb for its highly fragrant leaves. The dried/frozen leaves are used in the preparation of a variety of foods, as a condiment or spice, and as a source of aromatic essential oil for use in foods, flavors, and fragrances. Basil is also used and as a potted herb and as an ornamental bedding plant. The main type traded includes the large leaf highly aromatic Italian basil 'Italian Large leaf'. There are several other cultivars such as 'Genovese', 'Genovese Compact Improved', 'Nufar', etc., which differ in leaf shape and aroma that are of commercial importance in the fresh market industry (Simon, 1998).

Recently, there has been much research into the health benefits conferred by the essential oils found in basil. Scientific studies *in vitro* have established that compounds in basil oil have potent antioxidant, anticancer, antiviral, and antimicrobial properties (Almeida, 2007; Bozin, 2006; Chiang, 2005). Basil is used in traditional Chinese medicine to treat cardiovascular diseases including hypertension, which also showed the possible antihypertensive effects of basil extract in renovascular hypertensive rats (Umar, 2010). It is also traditionally used for supplementary treatment of stress, asthma and diabetes in India (Duke, 2008). Other traditional uses are in the treatment of headaches, coughs, diarrhea, constipation, warts, obesity, worms and kidney malfunctions (Simon et al., 1999). Basil also has religious significance in Hindu culture. Basil also contains estragole, a known carcinogen and teratogen in rats and mice. While human effects are currently unstudied, extrapolation using body weight from the rodent experiments

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Since basil is an herb, its production requires a stable and secure supply of high viability, vigorous seeds in order to maximize stands as well as the production and quality. Seed testing is the science of evaluating the quality of seeds to determine their value for planting. Though initially developed from field and garden seeds, seed testing is equally valuable for determining the seed quality of turf, flowers, herbs, shrubs, trees and native species. The term "seed quality" is loosely used to reflect the overall value of seeds for planting, viability is the most widely recognized aspect of seed quality. Other aspects of seed quality include physical purity, vigor, freedom from seed-borne diseases, contamination by noxious weeds, moisture content, and varietal or genetic identity and purity. Seed testing provides results that are of fundamental importance to enable producers to label seed quality information for the marketplace (Elias et al., 2012).

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Temperature is one of the most important factors for seed germination. The temperature responses of basil have not been widely studied. There is very little in the scientific literatures about the base, optimum and ceiling temperatures for basil seed germination. In one study, sweet basil germinated well at day/night temperature regimes between 18/13°C and 30/25°C , and germinated best at 25ºC with no saline stress (Ramin, 2006). Another study showed that germination of sweet basil seed was rapid and reached a high level, especially at temperatures between 21°C and 30°C, germinating to over 80% germination after four days. At all other temperatures either the percentage or the speed of germination was less (Putievsky, 1983).

Because of the wide range of seed germination temperatures, a thermo-gradient table is often used to test seed germination at many temperatures simultaneously. They are much more convenient and time saving to use for testing multiple temperature responses than growth chambers or incubators, which only can provide assessment of one temperature at one time. Thermo-gradient tables have been widely used to evaluate germination responses to temperature for a number of different species including trees, ornamental plants, grasses, vegetables crops, and agronomic crops (Orozco-Segovia, 1996; Schwember, 2005; Medany, 2007).

Plant mucilage is produced by many different plant species and by different types of plant tissues including seeds, leaves and roots (van Rheede van Oudtshoorn and Van Rooyen, 1999). Mucilage likely has several different functions, which may help plants store water (especially in succulent species) (Landrum, 2002). Mucilage may lubricate growing roots as they penetrate soil (Guinel and McCully, 1986), and may trap prey in carnivorous plants (Vintejoux, 2000).

The production of hydrophilic mucilage by the seed coat or pericarp during hydration is a common adaptation in angiosperms, known as myxodiaspory. The mucilage is composed primarily of pectins and hemicelluloses that undergo substantive swelling upon hydration. The ability to synthesize mucilage is a notable feature of dispersal (fruit and seeds) in many flowering plants (Western, 2012).

Double fertilization in angiosperms leads not only to embryogenesis and development of the endosperm, but also the differentiation of surrounding ovule integuments that become the seed coat. The coat has evolved special functions through time to protect the embryo, facilitate dispersal, and support germination. The specialized structures of seed coats may include cell walls impregnated with impermeable polymers that provide mechanical reinforcement (Western,

2012). Another common specialization is the deposition of hydrophilic, pertinacious mucilage in the seed coat epidermis. Mucilage may also be produced by pericarp tissues that surround the embryo in propagules that include ovary tissue (i.e. fruit) as part of the dispersal unit. Seed coat or pericarp mucilage is found in a wide range of species (Grubert, 1974; 1981). The production of seed mucilage is known as myxospermy, occurs in a broad range of plants, including Acanthaceae, Brassicaceae, Liaceae, and Plantaginaceae. While the fruit mucilage is called myxocarpy, occurs in family of Asteraceae, Lamiaceae and Poaceae (Grubert, 1974; Fahn, 1979; Grubert, 1981; Ryding, 2001; Kreitschitz, 2009).

Mucilage is deposited in the apoplast of epidermal cells during differentiation of the seed coat or pericarp, and is released during seed hydration to form a water-containing, gel-like capsule surrounding the seed (Western, 2012). Mucilage production by seed dispersal unit (seed coat or pericarp) has been proposed to play a number of roles, including facilitation of seed hydration, regulation of germination by affecting oxygen entry into seeds, and mediation of seed dispersal through adhesion to soil or animal vectors (Grubert, 1974; Fahn, 1982; Ryding, 2001; Kreitschitz, 2009; Deng, 2012). For example, the intact achenes of *Aytenisia sphaerocephala* (Asteraceae), which contain mucilage in their pericarp, had a higher germination percentage than that of demucilaged achenes (Yang et al., 2010). It has also been proposed that seeds which produce mucilage improve contact and adhesion to soil particles (Grubert, 1974).

Sweet basil produces a mucilaginous fruit. The outer pericarp (or outer epidermis) of basil seeds quickly swells into a gelatinous mass when soaked in water (Azoma and Sakamoto, 2003). In many Asian countries, the mucilage and seeds of sweet basil are bottled as a popular drink. The sweet basil seeds are also sold as a diet food to make drinks because the mucilage tends to fill the stomach easily reducing the craving for food. Therefore, basil seed mucilage is of interest not only for its effects on seed germination but also as a human food as well.

The sweet basil fruit mucilage is similar to *Arabidopsis* mucilage, but contains arabinoxylan and glucomannan (Anjaneyalu et al., 1983; Khan et al., 1987; Razavi et al., 2009). The polysaccharides extracted from basil seeds by cold water extraction and alcohol precipitation, have been reported to have two major fractions: (i) an acid-stable core glucomannan (43%) having a ratio of glucose to mannose 10:2, and (ii) a (1→4)-linked xylan (24.3%) having acidic

side chains at C-2 and C-3 of the xylosyl residues in the acid-soluble portion. Also, a minor fraction of glucan (2.3%) as a degraded cellulose material has been reported (Razavi et al. 2009).

Fig. 1. Sweet basil plants.

Fig. 2. Basil Production at Shenandoah Growers Inc. near Harrisonburg, Virginia (Photo by Gregory E. Welbaum, 2012).

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Chapter 2

Seed Germination Performance of Sweet Basil (*Ocimum basilicum* L.) Over a Broad Range of Temperatures

Abstract

Sweet basil (*Ocimum basilicum* L.) is a warm season herb usually propagated from seeds. Establishment of basil is difficult as seed germination may be limited, particularly during field seeding at cold soil temperatures. The germination of six cultivars ('Italian Large Leaf', 'Italian Large Leaf' 35X, 'Nufar', 'Genovese', 'Genovese Compact Improved' and 'Aroma 2') of sweet basil seeds were tested on a one dimensional thermo-gradient table over temperatures ranging from 0 to 50°C. At temperatures below 20°C, germination among cultivars was more variable and the mean time to germination (MTG) increased to greater than 25 days for some cultivars. Germination declined sharply and had a sudden termination at high temperatures above 40°C for all six cultivars. There were statistical differences among the cultivar base temperatures, which ranged between 10.1 and 13.3°C. The optimal and ceiling temperatures for germination were similar and did not differ statistically among the cultivars compared in this study. The average optimal temperature for all cultivars was 35 ± 0°C, while the average ceiling temperature was 43 ± 1.3°C. Stored seeds (> 5 years) had lower seed vigor and lower germination percentage, also lower ceiling temperature compared with the fresh seeds of the same cultivar ('Italian Large Leaf'), but the base temperatures were the same for both new and old seeds.

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for a number of different species including trees, ornamental plants, grasses, vegetables crops, and agronomic crops (Orozco-Segovia, 1996; Schwember, 2005; Medany, 2007).

The purposes of the present study were to determine the effect of temperature and seed age on germination percentage and rate of seeds from six cultivars of sweet basil. In order to obtain a better understanding of how temperature and seed age affect germination ability, the seed structure, and water relations of imbibition were also examined.

Materials and Methods

Plant material

Five popular sweet basil cultivars in North America were selected in spring 2011 from Johnny's Seeds Company (Fairfield, Maine) for study, included 'Italian Large Leaf', 'Nufar', 'Genovese', 'Genovese Compact Improved' and 'Aroma 2'. One additional cultivar 'Italian Large leaf' 35X, was provided by a California agent, which was an Italian Large Leaf type produced in 2006, and was reported to exhibit low seed vigor. 'Italian Large Leaf' is a higher yielding basil, with sweeter and less clove-like scent and taste compared with 'Genovese'. 'Nufar' is a disease (*Fusarium*) resistant Italian Large Leaf type for field, greenhouse, and hydroponic. 'Genovese' is a traditional Italian basil type with authentic flavor and appearance. 'Genovese Compact, Improved' is good for containers and pack sales and a preferred cultivar for greenhouses. It has similar flavor, appearance and leaf size as 'Genovese'. 'Aroma 2' is a disease (*Fusarium*) resistant Genovese type F1 hybrid, used for greenhouse or field production. Seeds of these five cultivars were purchased in March 2011. All seeds were sealed in plastic zip-bag or glass container and stored in refrigerator at 4°C with 9% water content until tested in a series of experiments from March 2011 through August 2012.

Thermo-gradient table

A one-dimensional, linear, thermo-gradient table consisting of a ¼" thick aluminum plate 1 wide × 1.2 m long was enclosed in an insulated case with covers (Fig. 3). Circulating baths were connected to square tubes welded to the underside of aluminum plate and circulated warm and cool ethylene glycol through opposite ends of the table to passively establish a continuous temperature gradient from one end of the table to the other. The temperature change across the gradient table was less than 1°C. Each experimental temperature reported was recorded with 2

WatchDog button-style data loggers (Spectrum Technologies, Inc., Temp 2K, B-Series), which are water resistant, can log temperatures ranging from - 10° to 85°C with accuracy of ± 1.1°C. Each data logger was set on top of the germination blotter paper among seeds inside a plastic box were germination experiments were conducted. Temperatures in germination experiments were recorded hourly, and averaged for each experimental temperature on the table.

Germination tests

The AOSA (The Association of Official Seed Analysts) defines seed germination as "the emergence and development of a seed embryo with those essential structures that, for kinds of seed in question are indicative of ability to produce a normal plant under favorable conditions." All the experiments were conducted base on standardized AOSA germination testing procedures for basil but using many different temperatures and allowing germination to continue until no more seeds germinated (Elias et al., 2012).

Four replications of 25 seeds per cultivar were placed on top of two thicknesses of germination blotter paper (Anchor Paper Co.) inside $11 \times 11 \times 4$ cm transparent covered plastic boxes (Hoffman Manufacturing Inc.) moistened with 15 ml distilled water, randomized within each temperature, and incubated in the dark. Germination was scored as radical emergence of ≥ 1 mm at 24-hour intervals until no further germination had occurred for three days (Jett and Welbaum, 1996). Seeds were removed from the boxes after radicle emerged and reached 1mm, and were recorded as germinated.

'Italian Large leaf' 35X and 'Nufar' were tested in one experiment in March-June 2011, germination temperatures of 5, 10, 14, 18, 23, 27, 32, 35, 39 and 43°C were maintained on the thermo-gradient table. 'Italian Large Leaf', 'Genovese', 'Genovese Compact Improved' and 'Aroma 2' were tested were tested in another separate experiment in September-December 2011, at germination temperatures of 7, 11, 13, 17, 19, 22, 26, 29, 32, 35, 38, 42 and 45°C.

Germination data analysis

The standard error of the mean was calculated from four replications of 25 seeds each to compare final germination percentages at each temperature. Mean time to germination (MTG) was calculated in days as $\sum (N_i T_i) / \sum (N_i)$, where N_i is the number of newly germinated seeds at

time T_i after imbibition. Germination rate (RT) was calculated as $GR = 1 \cdot t^{-1}$ or the inverse of MTG and expressed as seeds per day (Nerson and Paris, 1988).

The mean minimum or base temperature (T_b) for seed germination were determined by extrapolating plots of mean germination rate verses temperature (T) to the intercept on the abscissa (Gummerson, 1986). Since the plot of GR verses T, was not linear at high temperature, the mean maximum or ceiling temperature (T_m) was estimated using the temperature that reduced the germination percentage to 50% (Jett and Welbaum, 1996). The optimal temperature (T_o) for germination was determined as the combination of the highest germination percentage and the lowest MTG (highest GR).

Germination percentages, rates, MTG also the regression of germination rate from starting to maximum were plotted by SigmaPlot, 12.0. The base, optimal and ceiling temperatures were compared by analysis of variance (ANOVA) (SAS; SigmaPlot Analysis, 12).

Results and Discussion

Final germination percentages were similar and high for all cultivars at temperature 18 - 35 °C. The final maximum percentages of germination were greater than 90% for cultivars 'Italian Large Leaf' (Fig. 4A), 'Nufar' (Fig. 6A), 'Genovese Compact Improved' (Fig. 8A) and 'Aroma 2' (Fig. 9A), and 85% for cultivars 'Italian Large Leaf' 35X (Fig. 5A) and 'Genovese' (Fig. 7A). Germination percentage increased sharply from 7 - 14°C for all six cultivars, and remained high between 17 and 40°C (Fig. 10). Also MTG was relatively short between 17- 40°C (Fig. 11) what was the range. You can give a few examples with actual data here to illustrate the real values. At temperature 39 - 45°C (no germination observed at > 45°C for all cultivars), the germination percentages for all cultivars declined sharply from around 90% to 0 (Fig. 10). For stored low vigor seeds (cultivar 'Italian Large leaf' 35X), germination percentage was lower and did not occur at > 39°C (Fig. 5, 10). All other five fresh seeds cultivars still had > 60% germinated at 42°C (Fig. 10).

The seed vigor of all 6 cultivars was compared at different temperatures on the basis of mean time to germination (MTG). At low temperatures < 14°C, the MTG can be greater than 25 days (Fig 11). At temperatures > 27°C, almost all 2011 seeds germinated within 2 days. Stored low vigor seeds (cultivar ''Italian Large leaf' 35X, Fig. 5), took longer time to germinate (3.8 days at 23°C, 8.7 days at 18°C, 21.8 days at 14°C and 42.7 days at 10°C).

The GR (germination rate) increased linearly from 10°C to maximum at 35°C for all cultivars, and then declined sharply at higher temperatures. The GR at temperatures greater than maximum did not decline linearly. At most temperatures, the GR of stored seeds ('Italian Large leaf' 35X) was lower than the other five cultivars purchased in 2011. GR of 5 cultivars produced in 2011 were similar (Fig. 12). The base (T_b) temperatures for germination for 6 sweet basil cultivars were caculated by the regression equations (Fig. 13, Table 1). Analysis of variance (ANOVA) of base temperatures and germination rate (from the starting to maximum) slopes (Table. 2) shows, the differences in the mean values among the cultivar groups are greater than would be expected by chance, so there is a statistically significant difference ($P = < 0.001$) (Table. 3, 4). The optimal temperature (T_o) for germination was determined by finding the temperature where germination percentage was high and the MTG was low (or GR was high) across a range of temperatures. All 6 cultivars had optimal germination at 35°C (Fig. 4, 5, 6, 7, 8 and 9), no differences were detected (Table 1).

An accurate graphic determination of T_m was not possible because of the abrupt decline in germination percentages > 40°C. At temperature > 35°C, the germination rate is not linear. The T_m was estimated by comparing the temperatures that inhibited the germination percentage by 50% (Fig. 4A, 5A, 6A, 7A, 8A, 9A and 10). There was no significant difference among cultivars and no differences were detected among cultivars (Table 1).

Effects of temperature on sweet basil seed germination were in the range of those from previous studies (Putievsky, 1983; Ramin, 2006), where basil seed germinated between 13 to 30°C, to more than 80% after four days. Unlike the earlier work, this study characterized basil seed germination over a much wider range of temperatures. The thermo-gradient table was well suited to conducting this type of study because it allowed replicates of cultivars to be carried out over a wide range of temperatures simultaneously. One problem was that not all seeds in this study could be tested at the same time so the experiment was broken into two parts with some cultivars being tested on the gradient table first and a second group compared after the first experiment was completed four months later. This approach required that seeds of some cultivars be stored for longer periods than others. However, aging during the longer period of storage was likely not a problem because the seeds were all stored in sealed containers at low moisture content (9%) on a refrigerator (4 °C). Also there was no discernible difference in the germination performance between the early and late testing group. Therefore, we can conclude

the extra storage period caused by not being able to test all seeds in one experiment likely had no impact on the results of this study. Basil is a warm season frost sensitive crop and as anticipated germination was faster and germination percentages greater at higher temperatures. It was interesting that the base temperatures were relatively low, just above 10 °C, for a warm season crop that was obviously better acclimated to high temperatures. However, at low temperatures, germination was very slow, which creates a problem during field establishment because the seeds can be attacked by a wide range of pathogens before they can germinate and emerge.

Variations in base temperature have been the source of great debate of the past couple of decades. For many seed species in the absence of dormancy, germination rate increases linearly above a base temperature (T_b). The slopes of these lines are the reciprocal or inverse of the thermal times to germination $(1/\theta_T)$. As temperature increases above an optimum (T_o), the rate of germination increases to a ceiling temperature (T_m), which was also the case for the basil seeds in this study. T_b is often the same for all seeds in a given population, but $1/\theta_T$, and T_m vary among seeds in a normal distribution. The question is how much variation exists in T_b among different cultivars within a given species? The earlier work of Ellis and Butcher s indicated that within a species, the base temperature was a constant value genetically controlled. Ellis and Butcher's proposed that in the absence of dormancy, base temperature (T_b) is genotypically, rather than developmentally controlled (Ellis and Butcher, 1988). The current study contradicts this proposal because there were significant differences in base temperatures calculated among cultivars in the same species. These differences were likely not caused by dormancy because at high temperatures all seeds germinated rapidly. The calculation of base temperatures were based on graphic interpolation to a germination rate of zero so subtle differences in slope could affect base temperature calculations. However, the regression lines used to calculate the base temperature had six or more points and were immune to the influences of aberrant data points. Also each value was the average of four replications. Therefore, it appears that significant variation in base temperature exists among basil cultivars so that is may be possible through breeding to decrease the base temperature and improve low temperature germination performance. Other plant species have shown variation in base temperature as well. A number of other studies have shown that T_b may vary with environment and hormonal conditions (Welbaum et al., 1990). Storage reduced the estimated base temperature (T_b) for germination of both 40 and 60 DAA (days after anthesis) seeds by approximately 5°C (Welbaum and Bradford,

1991). Since we do not know the production history of the seeds tested in this study it is possible that variations in seed age at harvest could affect (T_b) as well and account for the variations observed. 'Italian large leaf' and 'Italian Large leaf' 35X were the same genotype, but seed were harvested in different years. The older seeds had lower seed viability and vigor, but the same T_b. The old 'Italian Large leaf' 35X seeds had lower germination percentage and longer MTG, larger SE, and lower slope of plot of the germination rate regression line compared with the fresh seeds (Fig. 14). The base temperature did not change with relatively long-term storage even though the vigor and viability decreased. However the thermal time to germination (θ_T) increased after long-term storage. Therefore base temperature is independent of seed quality and did not change with viability or vigor. Also the old seeds had lower ceiling temperature so performance at high temperatures was negatively affected. The optimal temperature for germination did not change for basil and was 35°C for all cultivars.

Conclusions

There is significant low temperature variation in basil seed germination. Some cultivars germinate better at low temperatures than others. The variation was independent of changes in seed quality since stored and aged seeds displayed the same base temperature as newly harvested seed lots of the same cultivar. Therefore changes in T_b may be genetic. The potential exists to improve the low temperature germination performance of basil seed through plant breeding and selection for low temperature performance.

At temperatures below 20°C, germination among cultivars was more variable, for both germination percentage and MTG. Germination declined sharply and had a sudden termination at high temperatures above 40°C for all six cultivars. Germination of all cultivars ceased at temperatures around 45°C. The base temperatures (T_b) for germination were statistically significant among cultivars and varied between 10.1 and 13.3°C. The optimal and ceiling temperatures were similar and did not vary among the cultivars compared in this study. The optimal (T_o) temperature was 35°C, and the ceiling temperature (T_m) was 43.0 ± 1.3°C and there were no significant difference among cultivars (Tab. 7). Low vigor seeds ('Italian Large Leaf' 35X) had lower ceiling temperature, but base and optimal temperature did not vary.

Table. 1. Base (T_b), ceiling (T_m) and optimal (T_o) temperatures for germination of 6 sweet basil cultivars.

Cultivar	T_b (°C)	T_m (°C)	T_o (°C)
'ILL'	11.9	43.6	35
'ILL' 35X	12.3	40.4	35
'Nufar'	9.8	44.1	35
'Genovese'	10.9	43.4	35
'GCI'	10.8	43.3	35
'Aroma 2'	13.2	43.3	35
Mean		43.0 ± 1.3	35
	**	NS	NS

All means were calculated graphically from data in Fig 4-9 from 4 replications of 25 seeds each for seed germination.

NS, ** Nonsignificant or significant at P = 0.01.

Table. 2. Mean separation of base temperatures (T_b) and germination rate (from the starting to maximum) slopes of each cultivar.

Cultivar	T_b (°C)	Slope
'ILL'	11.9 ± 0.3 [b]	0.0340 ± 0.0017 [b]
'ILL' 35X	12.3 ± 0.9 [b]	0.0250 ± 0.0015 [c]
'Nufar'	9.8 ± 0.6 [d]	0.0373 ± 0.0021 [a]
'Genovese'	10.9 ± 0.3 [c]	0.0400 ± 0.0022 [a]
'GCI'	10.8 ± 0.4 [c]	0.0377 ± 0.0020 [a]
'Aroma 2'	13.2 ± 0.4 [a]	0.0386 ± 0.0020 [a]
	**	**

Means were calculated graphically from data in Fig. 13 from 4 replications of 25 seeds each for seed germination. Means are significantly different by LSD$_{0.05}$ when separated by different letters within columns.

[**] Significant at P = 0.001.

Table. 3. One Way Analysis of Variance of base temperatures (T_b) (°C).

Source of Variation	DF	SS	MS	F	P
Between Groups	5	30.608	6.122	20.774	<0.001
Residual	18	5.304	0.295		
Total	23	35.913			

DF, degree of freedom; SS, sum of square; MS, mean square; F, F value; P, P value.

The differences in the mean values among the cultivar groups are greater than would be expected by chance; there is a statistically significant difference (P = < 0.001).

Table. 4. One Way Analysis of Variance of germination rate (from the starting to maximum) slopes.

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.00060033	0.00012007	31.7	<0.001
Residual	18	0.0006818	0.00000379		
Total	23	0.0006651			

DF, degree of freedom; SS, sum of square; MS, mean square; F, F value; P, P value.

The differences in the mean values among the cultivar groups are greater than would be expected by chance; there is a statistically significant difference (P = < 0.001).

Fig. 1. Sweet basil plants.

Fig. 2. Basil Production of Shenandoah Growers Inc. near Harrisonburg, Virginia (Photo by Gregory E. Welbaum, 2012).

Fig. 3. One-dimensional, linear, thermo-gradient table (top) with two circulating bathes (bottom).

Fig. 4. 'Italian Large Leaf'. (A) Germination percentage; (B) Mean time to germination (MTG); (C) Germination rate (GR); (D) Germination rate from the starting to maximum with regression, the regression equations is y = - 0.319 + 0.0254 x (r² = 0.988).
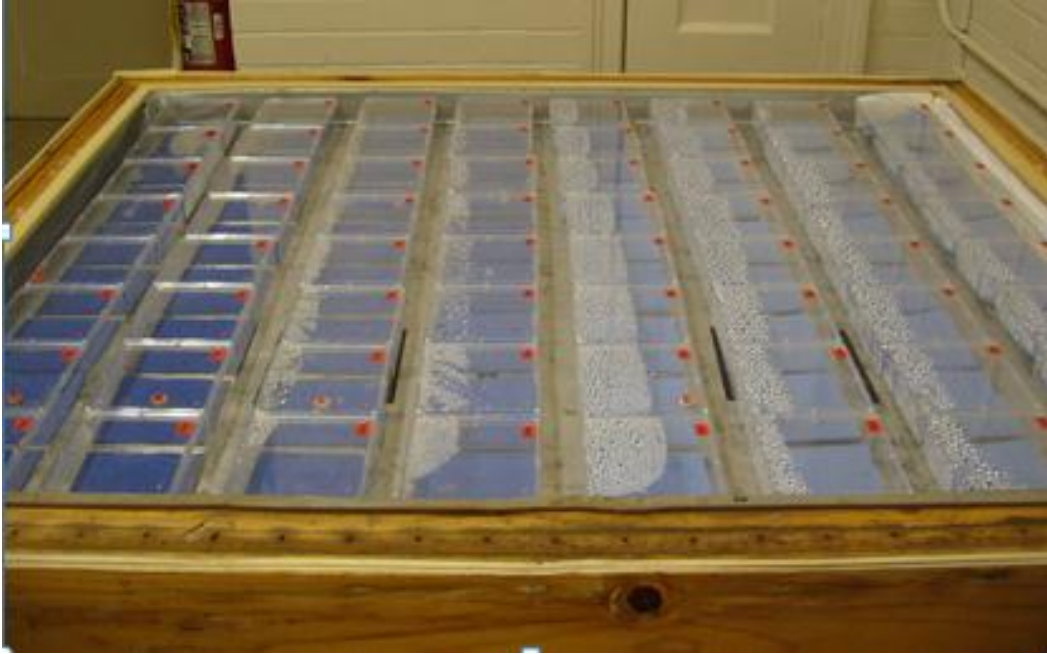
Fig. 5. 'Italian Large Leaf' 35X. (A) Germination percentage; (B) Mean time to germination (MTG); (C) Germination rate (GR); (D) Germination rate from the starting to maximum with regression, the regression equations is $y = - 0.319 + 0.0254 \, x$ ($r^2 = 0.988$).
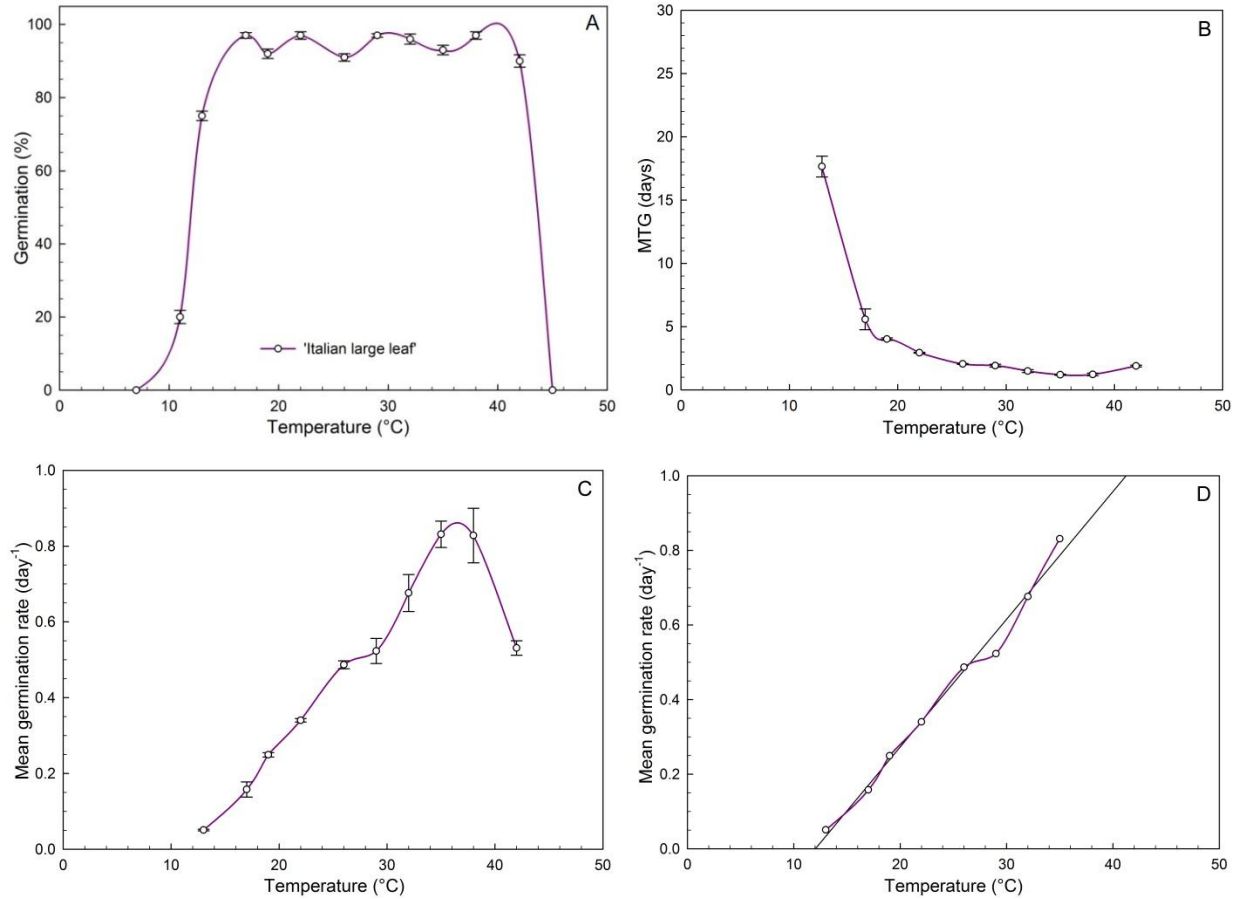
Fig. 6. 'Nufar'. (A) Germination percentage; (B) Mean time to germination (MTG); (C) Germination rate (GR); (D) Germination rate from the starting to maximum with regression, the regression equations y = – 0.394 + 0.0390 x (r² = 0.983).
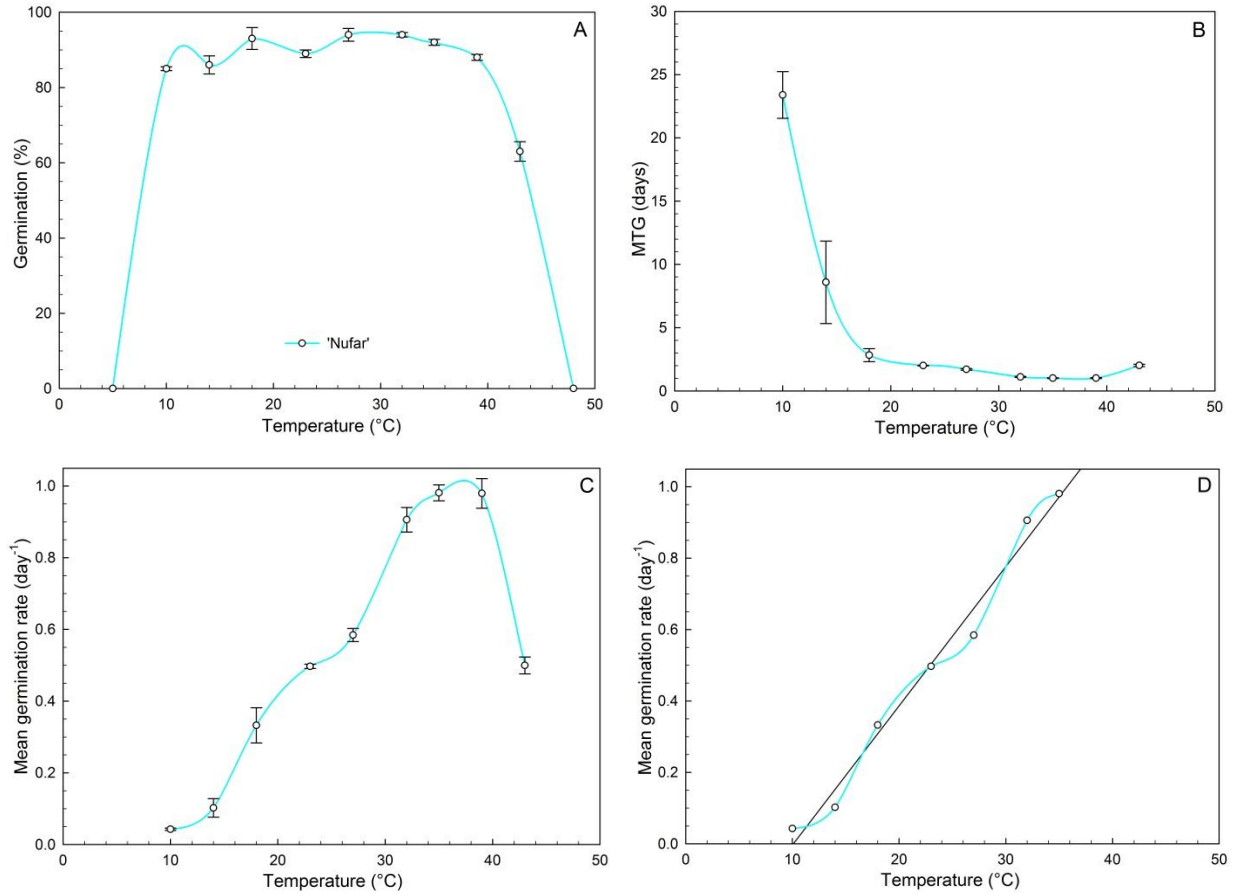
Fig. 7. 'Genovese'. (A) Germination percentage; (B) Mean time to germination (MTG); (C) Germination rate (GR); (D) Germination rate from the starting to maximum with regression, the regression equations is y = – 0.460 + 0.0412 x (r² = 0.978).
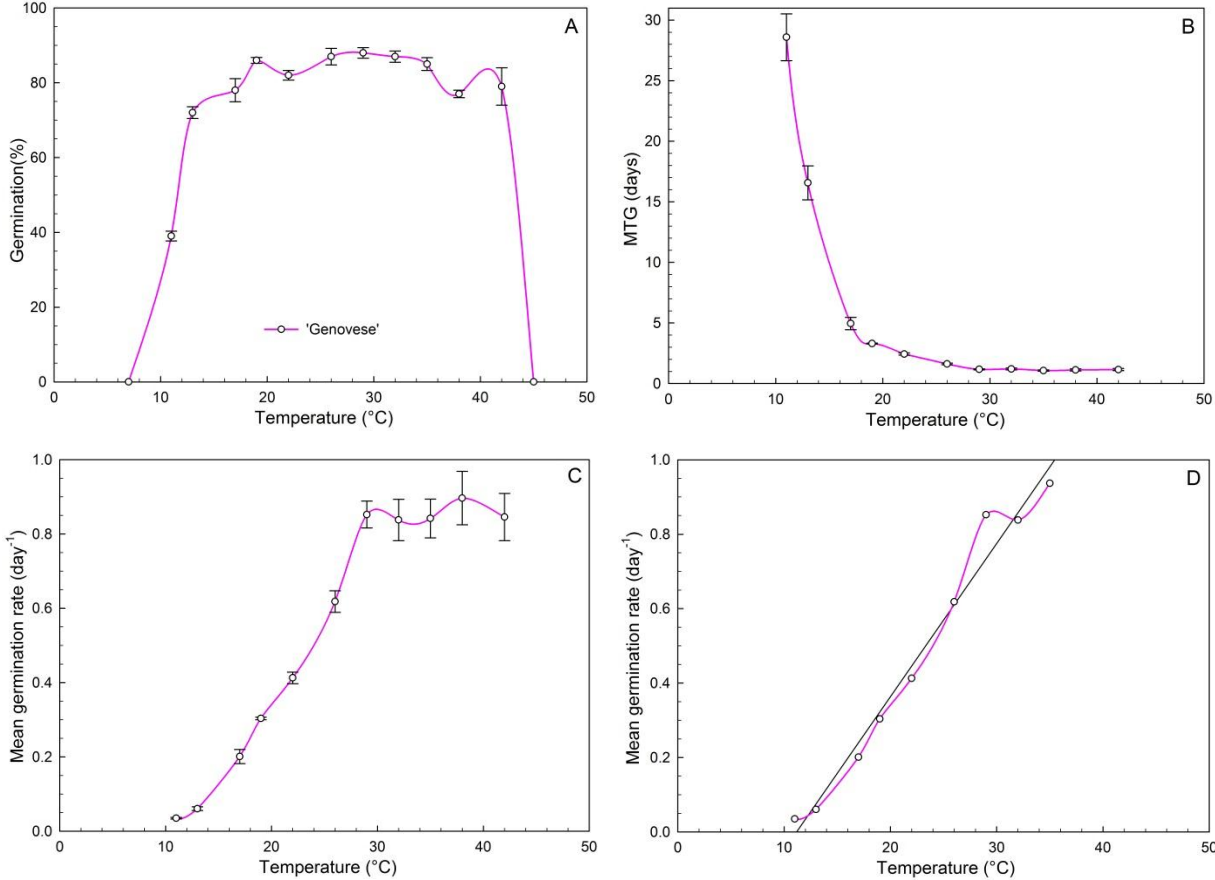
Fig. 8. 'Genovese Compact, Improved'. (A) Germination percentage; (B) Mean time to germination (MTG); (C) Germination rate (GR); (D) Germination rate from the starting to maximum with regression, the regression equations is $y = -0.425 + 0.0390 \, x$ ($r^2 = 0.983$).
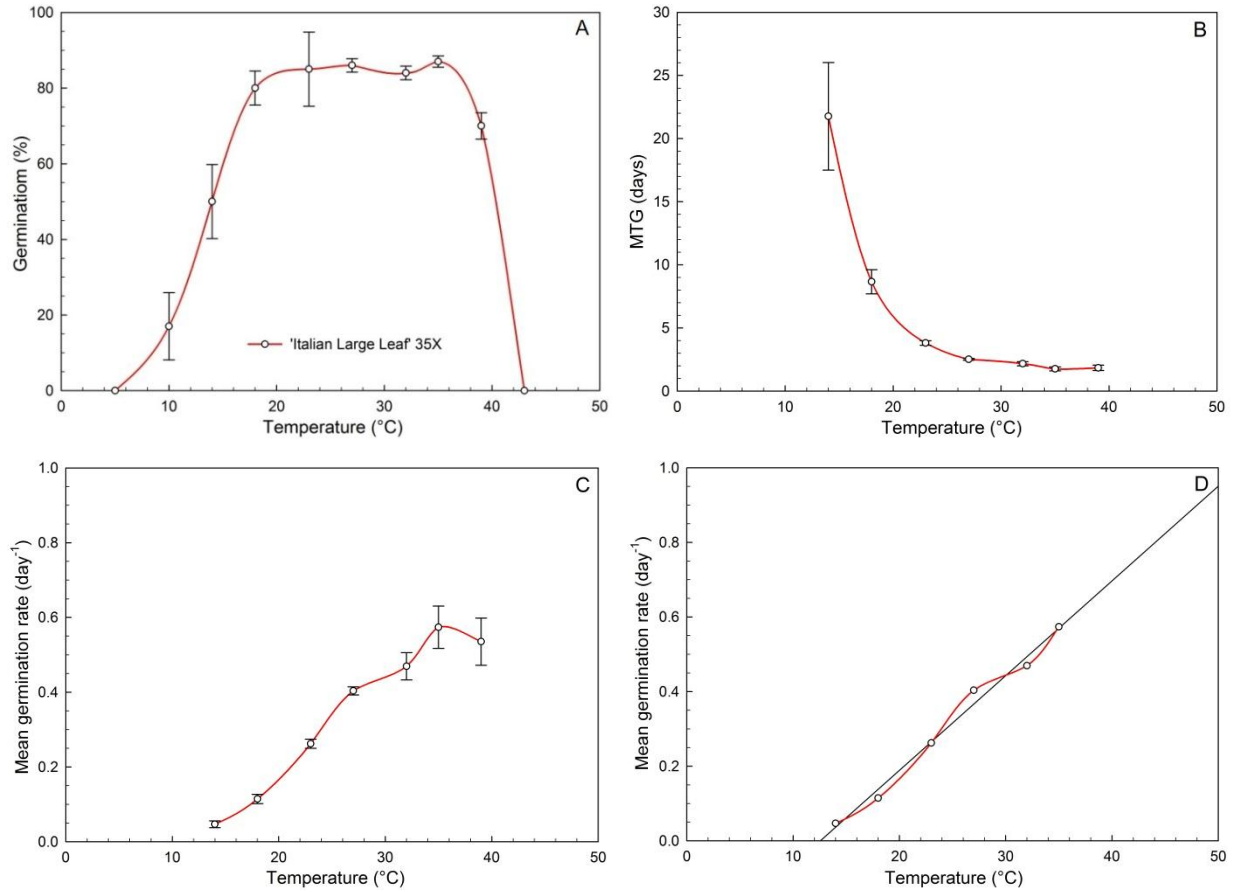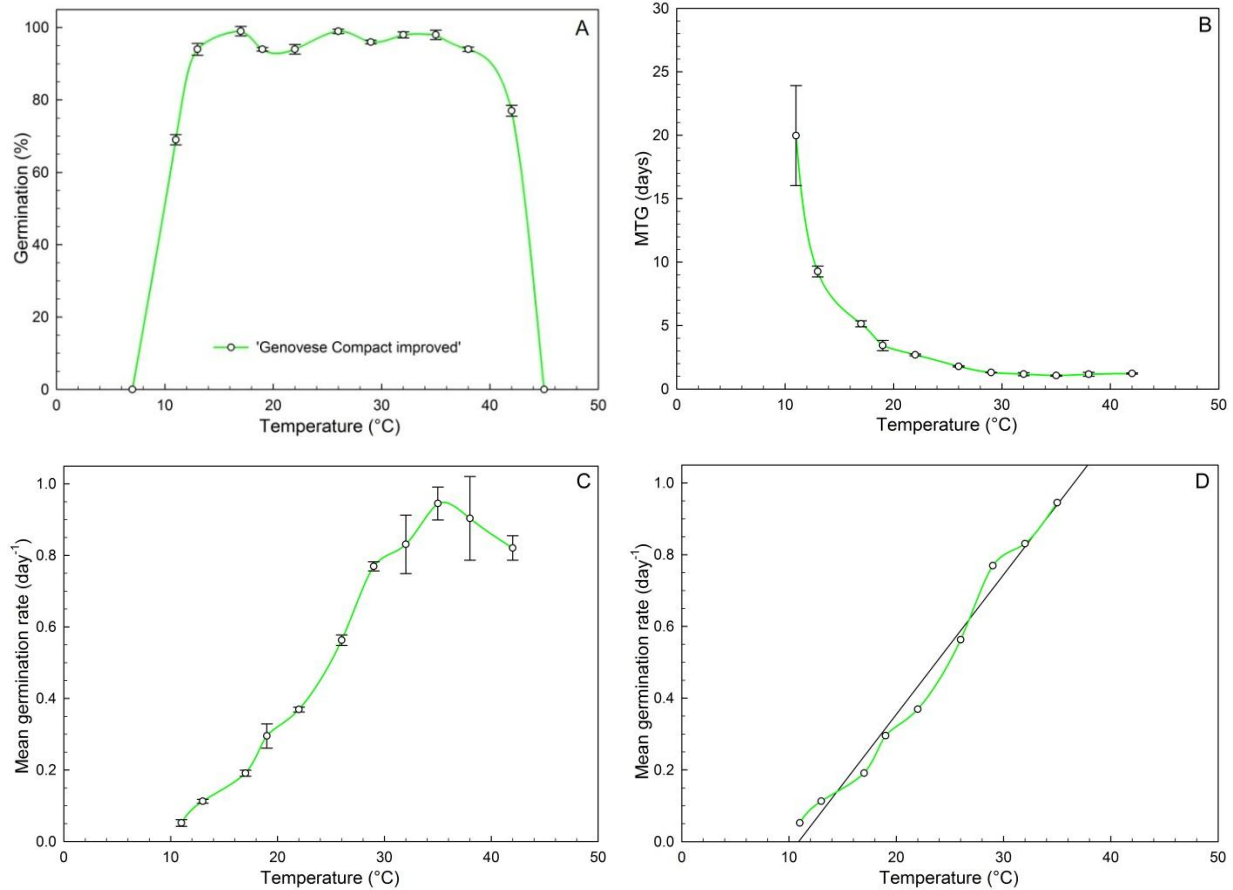
Fig. 9. 'Aroma 2'. (A) Germination percentage; (B) Mean time to germination (MTG); (C) Germination rate (GR); (D) Germination rate from the starting to maximum with regression, the regression equations is y = -0.511 + 0.0386 x (r² = 0.972).

Fig. 10. Germination percents of all 6 cultivars.
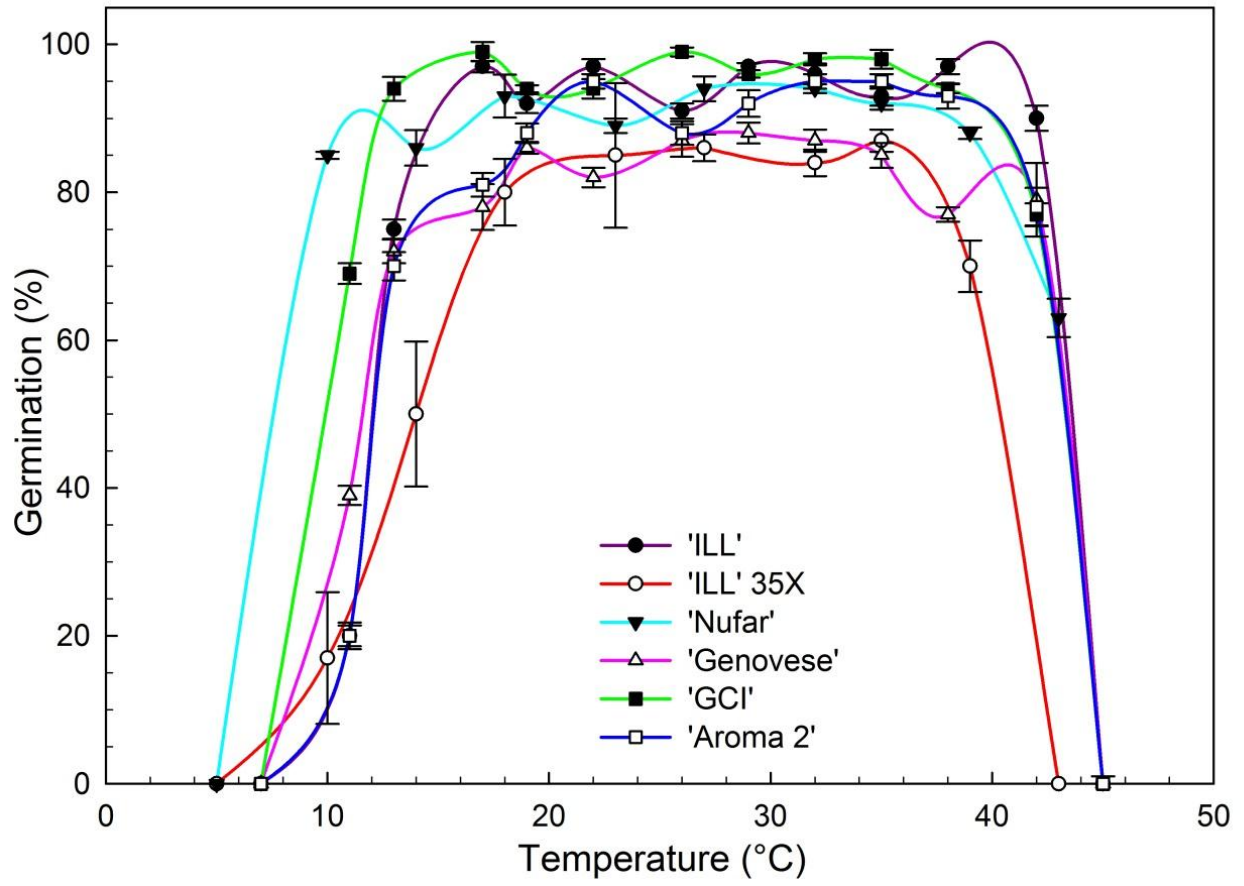
Fig. 11. MTG (Mean time to germination) of all 6 cultivars: $\sum (N_i T_i)/ \sum (N_i)$, where N_i is the number of newly germinated seeds at time T_i after imbibition.
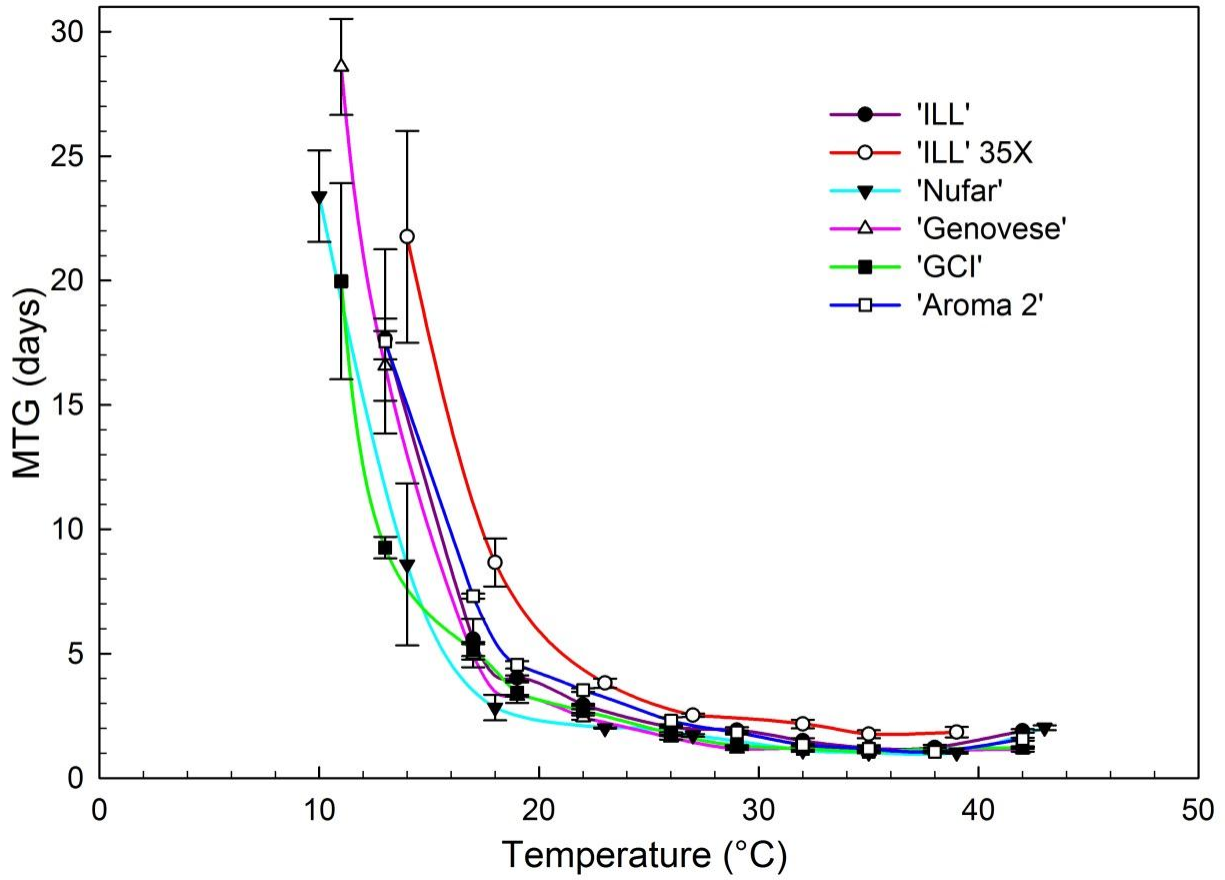
Fig. 12. Germination rate (GR) of all 6 cultivars.
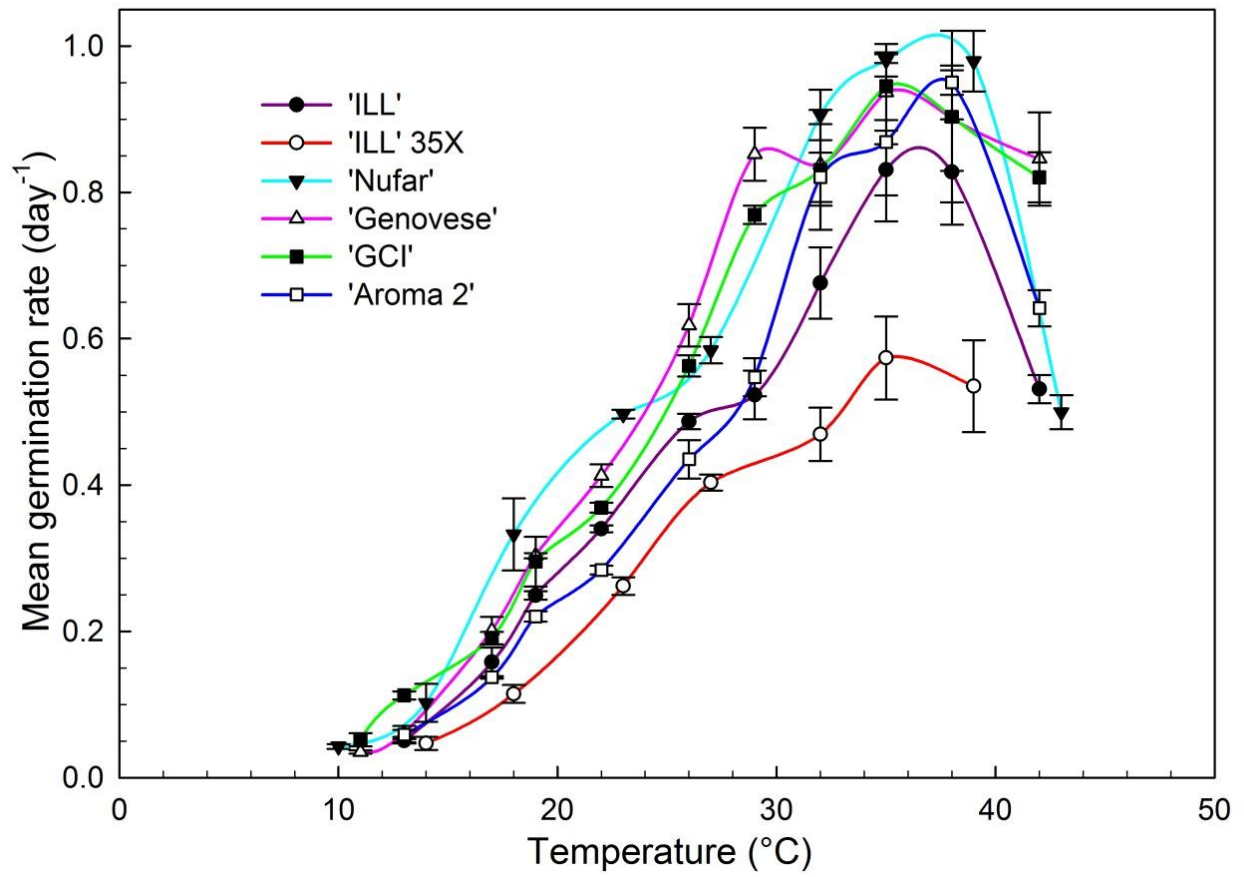
Fig. 13. Germination rate over the linear regression range of temperatures for all 6 cultivars. The regression equations are y = - 0.319 + 0.0254x (r² = 0.988, 'Italian Large Leaf', solid line); y = - 0.319 + 0.0254 x (r² = 0.988, 'Italian Large Leaf' 35X, dotted line); y = - 0.394 + 0.0390 x (r² = 0.983, 'Nufar', short dash line); y = - 0.460 + 0.0412 x (r² = 0.978, 'Genovese', dash-dot-dot line); y = - 0.425 + 0.0390 x (r² = 0.983, 'Genovese Compact Improved', medium dash line); y = - 0.511+ 0.0386 x (r² = 0.972, 'Aroma 2', dash-dot line).
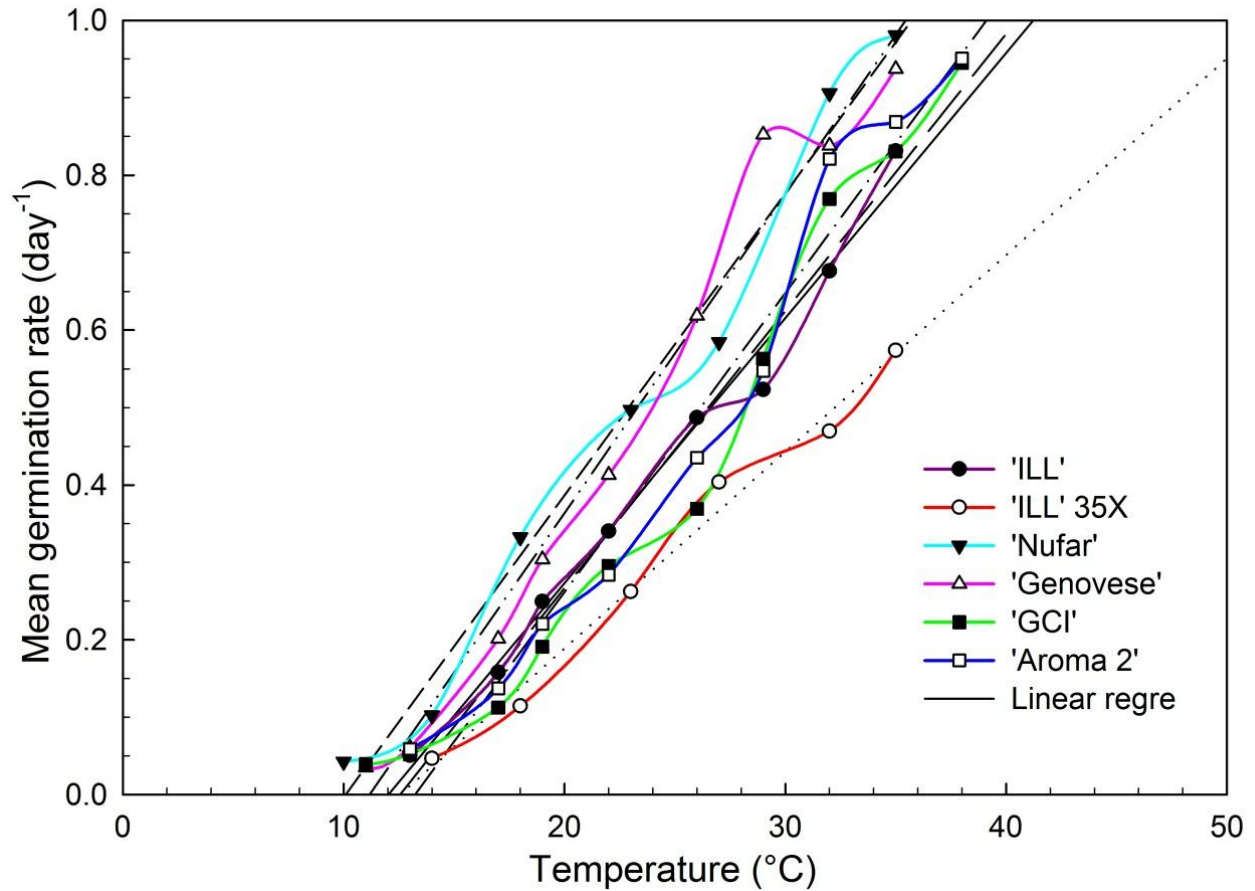
Fig. 14. Comparisons of 2 seed lots of 'Italian large leaf' stored for different lengths of time. (A) Germination percentage; (B) Mean time to germination (MTG); (C) Germination rate (GR); (D) Germination rate from the starting to maximum with regression, th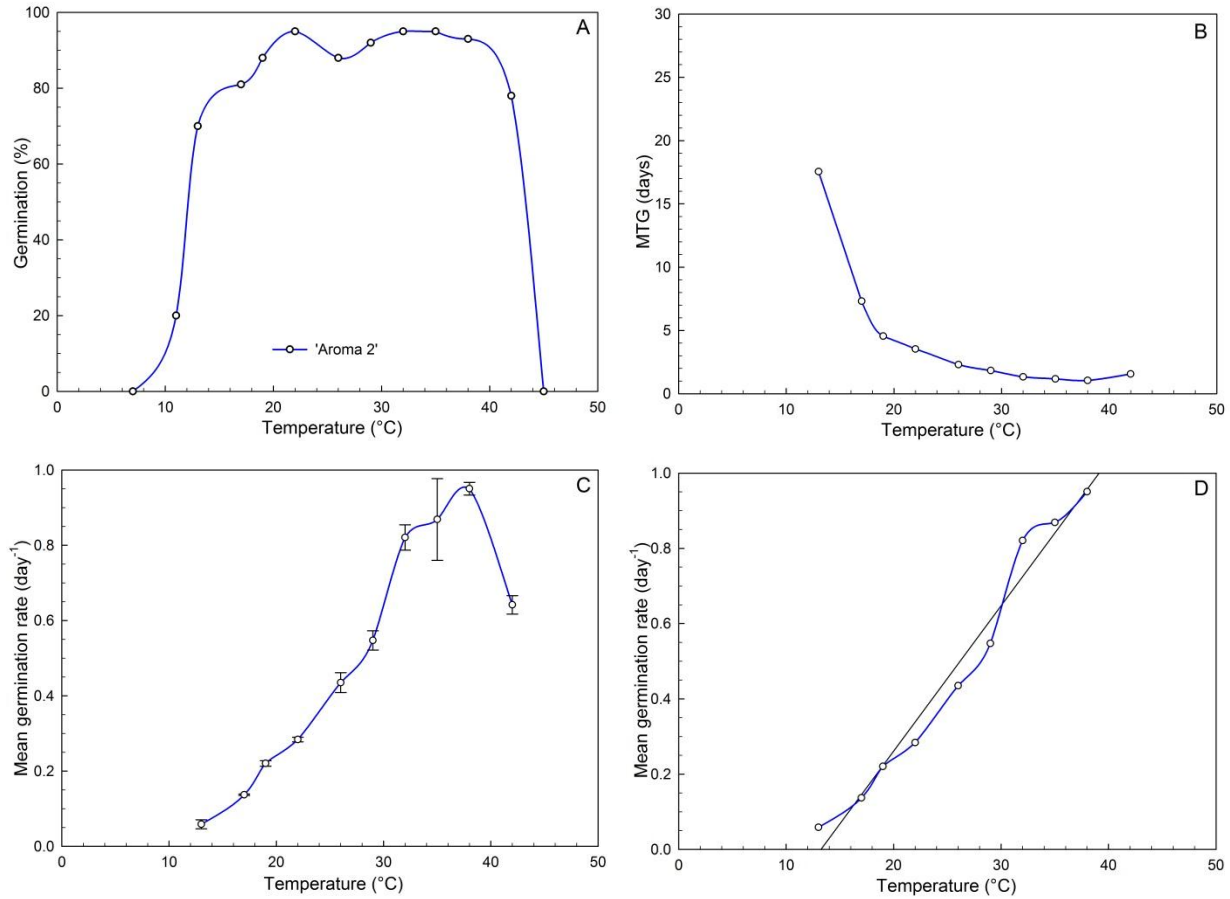e regression equations are y = – 0.319 + 0.0254 x (r² = 0.988, 'Italian Large Leaf' 35X), y = – 0.319 + 0.0254 x (r² = 0.988, 'Italian Large Leaf').
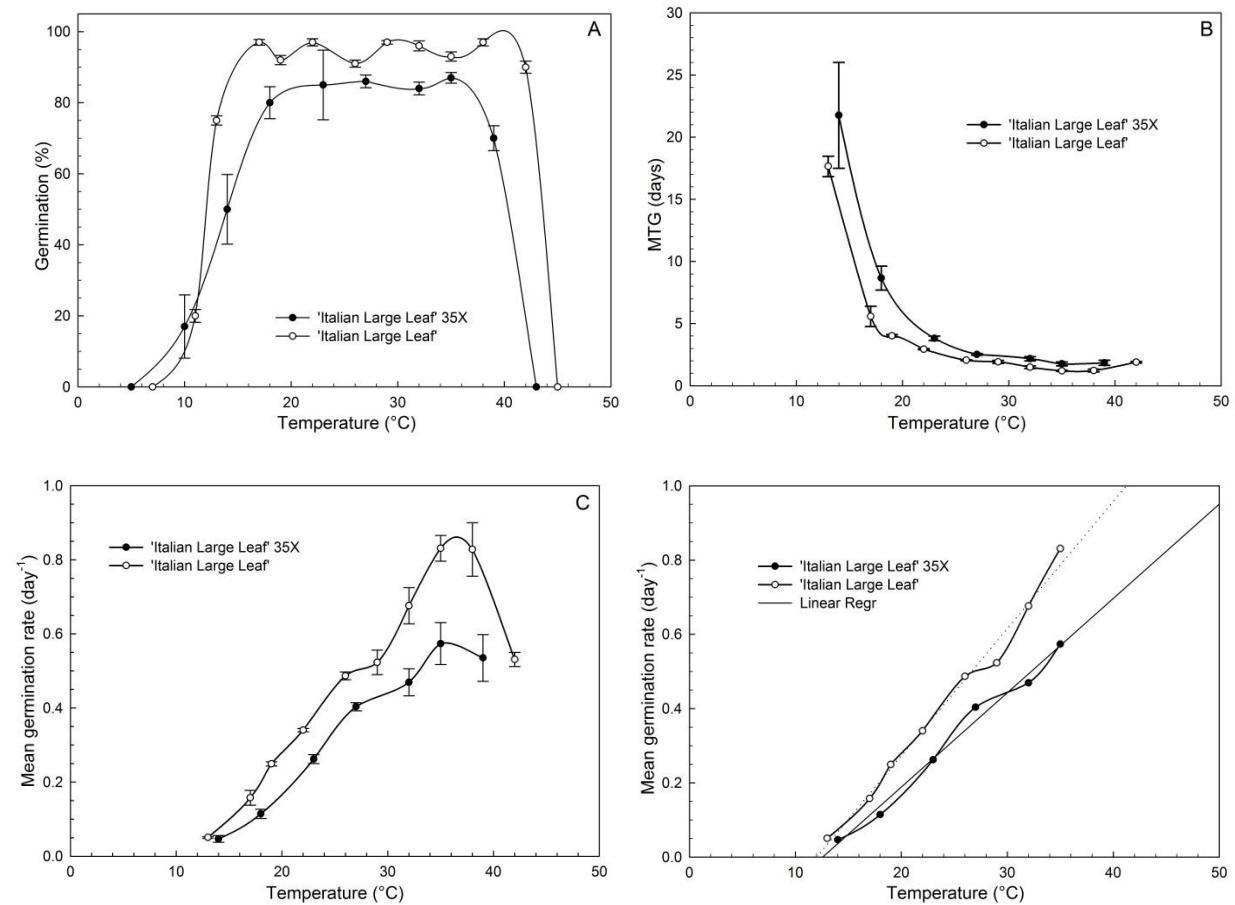
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Chapter 3

The Production and Function of Mucilage by Sweet Basil (*Ocimum basilicum* L.) Seeds

Abstract

Sweet basil (*Ocimum basilicum* L.) seeds produce a thick layer of mucilage around the pericarp within minutes after hydration. Mucilage is most prevalent among plant species adapted to surviving in arid sandy soils, though its significance in determining ecological fitness is unclear. The mucilage produced by seeds is reported to be composed of cell-wall polysaccharides that are deposited in testa pericarp cells during development. In this study, sweet basil seeds were examined using light and environmental scanning electron microscopy. The mucilage of basil seeds is held together by columnar structures that unfolded from the pericarp and helped hold and stabilize the mucilage to the outer surface. The mucilage was removed using diluted hydrochloric acid to compare performance of seeds with and without mucilage. Mucilage removal did not inhibit seed germination under ideal laboratory conditions but decreased the water content of seeds significantly. The water content of intact seeds was almost 4 times greater than seeds without mucilage. Mucilage enabled seeds cling to an incline board set to a steeper angle than seeds without mucilage. The fully hydrated seeds approached zero water potential, so the mucilage did not prevent seeds from fully hydrating. Soil (media) germination testing showed the seeds with mucilage had higher germination percentage than the seed without mucilage on several different types of media. Seeds with mucilage also had higher survival percentages after 10 days on different types of media. Basil seeds mucilage acts as a reservoir to hold loosely bound water at high water potential so it is available for seed germination and early seedling development.

Introduction

Plant mucilage is produced by many different plant species and by different types of plant tissues including seeds, leaves and roots (van Rheede van Oudtshoorn and Van Rooyen, 1999). Mucilage likely has several different functions, which may help plants store water (especially in succulent species) (Landrum, 2002). Mucilage may lubricate growing roots as they penetrate soil (Guinel and McCully, 1986), and may trap prey in carnivorous plants (Vintejoux, 2000).

The production of hydrophilic mucilage by the seed coat or pericarp during hydration is a common adaptation in angiosperms, known as myxodiaspory. The mucilage is composed primarily of pectins and hemicelluloses that undergo substantive swelling upon hydration. The ability to synthesize mucilage is a notable feature of dispersal (fruit and seeds) in many flowering plants (Western, 2012).

Double fertilization in angiosperms leads not only to embryogenesis and development of the endosperm, but also the differentiation of surrounding ovule integuments that become the seed coat. The coat has evolved special functions through time to protect the embryo, facilitate dispersal, and support germination. The specialized structures of seed coats may include cell walls impregnated with impermeable polymers that provide mechanical reinforcement (Western, 2012). Another common specialization is the deposition of hydrophilic, pertinacious mucilage in the seed coat epidermis. Mucilage may also be produced by pericarp tissues that surround the embryo in propagules that include ovary tissue (i.e. fruit) as part of the dispersal unit. Seed coat or pericarp mucilage is found in a wide range of species (Grubert, 1974; 1981). The production of seed mucilage is known as myxospermy, occurs in a broad range of plants, including Acanthaceae, Brassicaceae, Liaceae, and Plantaginaceae. While the fruit mucilage is called myxocarpy, occurs in family of Asteraceae, Lamiaceae and Poaceae (Grubert, 1974; Fahn, 1979; Grubert, 1981; Ryding, 2001; Kreitschitz, 2009).

Mucilage is deposited in the apoplast of epidermal cells during differentiation of the seed coat or pericarp, and is released during seed hydration to form a water-containing, gel-like capsule surrounding the seed (Western, 2012). Mucilage production by seed dispersal unit (seed coat or pericarp) has been proposed to play a number of roles, including facilitation of seed hydration, regulation of germination by affecting oxygen entry into seeds, and mediation of seed dispersal through adhesion to soil or animal vectors (Grubert, 1974; Fahn, 1982; Ryding, 2001; Kreitschitz, 2009; Deng, 2012). For example, the intact achenes of *Aytenisia sphaerocephala* (Asteraceae), which contain mucilage in their pericarp, had a higher germination percentage than that of demucilaged achenes (Yang et al., 2010). It has also been proposed that seeds which produce mucilage improve contact and adhesion to soil particls (Grubert, 1974).

Sweet basil produces a mucilaginous fruit. The outer pericarp (or outer epidermis) of basil seeds quickly swells into a gelatinous mass when soaked in water (Azoma and Sakamoto, 2003).

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In many Asian countries, the mucilage and seeds of sweet basil are bottled as a popular drink. The sweet basil seeds are also sold as a diet food to make drinks because the mucilage tends to fill the stomach easily reducing the craving for food. Therefore basil seed mucilage is of interest not only for its effects on seed germination but also as a human food as well.

The sweet basil fruit mucilage is similar to *Arabidopsis* mucilage, but contains arabinoxylan and glucomannan (Anjaneyalu et al., 1983; Khan et al., 1987; Razavi et al., 2009). The polysaccharides extracted from basil seeds by cold water extraction and alcohol precipitation, have been reported to have two major fractions: (i) an acid-stable core glucomannan (43%) having a ratio of glucose to mannose 10:2, and (ii) a (1→4)-linked xylan (24.3%) having acidic side chains at C-2 and C-3 of the xylosyl residues in the acid-soluble portion. Also, a minor fraction of glucan (2.3%) as a degraded cellulose material has been reported (Razavi et al. 2009).

In this study, sweet basil seed mucilage was evaluated by comparing germination performance of seeds with and without mucilage, visualization of seeds using scanning environmental electron and light microscopy, and a comparison of seed-water relations to figure out the mucilage function.

Materials and methods

Plant material

Three cultivars are selected from Johnny's Seed Company (Fairfield, Maine): 'Italian Large Leaf', 'Genovese' and 'Aroma 2'. 'Italian Large Leaf' is a higher yield basil cultivar, with sweeter and less clove-like scent and taste compare with 'Genovese'. 'Aroma 2' is F1 hybrid, a disease (*Fusarium*) resistant of Italian traditional Genovese type, adapted to greenhouse or field production.

Mucilage removal of sweet basil seeds

Both mechanical and chemical techniques were used to remove the sweet basil pericarp mucilage. For mechanical removal, hand scarification with sandpaper was used to remove mucilage.

For chemical removal, 12.6% diluted hydrochloric acid was used. Concentrated hydrochloric acid (Fisher Chemical, 37.71%) and distilled water was mixed at 1:2 volume ratios

of. Basil seed were added to the diluted acid and stirred for 5 minutes; filtered through a 1mm sieve, washed with running water, and dried at low temperature (25°C).

Light microscopy

DinoXcope (Dino-Lite Digital Microscope) was used to view seeds and to record seed hydration videos. Seed were stained with 0.1% Toluidine blue, which stains nucleic acids blue and polysaccharides purple and also increases the sharpness of thin sections of tissue samples. A light microscope (Olympus SZX16) was used to view thin sections of basil seed tissues. Seed tissues were prepared for thin sectioning as follows: (1) Seeds were placed in 10% formalin. (2) Samples were dehydrated in tissue processor in formalin, 70% alcohol, 80% alcohol, 3 changes of 95% alcohol, 3 changes of 100 % alcohol, 2 changes of changes xylene, and 4 changes of liquid paraffin wax. The tissue processor steps were set for 1 hour duration in all solutions except paraffin, which was 30 minutes each. (3) Dehydrated seed samples were imbedded and molded into a paraffin block. (4) The samples in paraffin blocks were cut with a rotary microtome into 4 micron thin-sections. The thin sections were floated on water and picked up on microscope slides. Slides were dried at 60°C using a blower for 20 minutes to melt the paraffin, section containing the sample tissue so that it would adhere to the slide and remove excess water. (5) Samples were deparaffinized in 2 changes of xylene for 3 minutes each. Slides were next rehydrated in 95% alcohol, 2 changes for 3 minutes each, and then rinsed in running tap water on an automated stainer. (6) Slides were next rinsed in distilled water and (7) stained in 0.002% Toludine blue for 4 minutes and dehydrated in xylene with 95%, 100% alcohol, and 95% xylene. (8) The sample was protected with cover slip attached with a resinous mounting media. This makes the slide permanent for viewing by light microscopy.

ESEM (Environmental Scanning Electronic Microscopy) (Manufacture: FEI, Instrument: QNANTA 600F) was used to visualize the surface structure of dry, intact seeds, demucilaged seeds and dead seeds that do not produce mucilage or germinate. The mucilage surrounding hydrated seeds was also observed. The dry intact, demucilaged and dead seeds were sputter coated with Au/Pd (10nm thickness; Cressington Sputter Coater, 208HR) to increase image resolution.

Hydration and dehydration time course of sweet basil seeds

A hydration time course was created by weighing, four replicates of 50 seeds of two sweet basil cultivars 'Italian Large Leaf' and 'Aroma 2', both with and without mucilage. To hydrate the seed samples, each of the four replicates were placed on top of a $5 \times 5 \times 2$ cm fully hydrated filter paper sheets (Whatman, Qualitative 1) in a $11 \times 11 \times 4$ cm transparent covered plastic boxes, on top of two thicknesses of germination blotter paper (Anchor Paper Co.) at 22°C. The blotter paper sheets were hydrated with 15 ml of water so that excess water collected in the corners of each box. Each filter paper replicate with seeds were remove with forceps and weighed on a balance every 15 minutes for the first hour, then 30 minutes for the duration of the experiment until the first radicle emerged. The weight of the seeds was determined by subtracting the weight of the hydrated filter paper and averaged across replications. The fully hydrated seeds were placed in a small aluminum plate, and dried in an over at 103°C for >16 hours to determine the dry weight of each seed sample.

To create a dehydration time course, four replicates of 50 seeds each of two sweet basil cultivars 'Italian Large Leaf' and 'Aroma 2', both with and without mucilage, were fully hydrated at 22°C, on top of a $5 \times 5 \times 2$ cm filter paper box in $11 \times 11 \times 4$ cm transparent covered plastic boxes, on top of two thicknesses of fully hydrated germination blotter paper (Anchor Paper Co.), until the weight plateaued. Each replicate of fully hydrated seeds were transferred to a small aluminum plate, and dried in 0.5 m^3 drying oven maintained at 25°C. The weight of each replicate was measured every 30 minutes till the weight stabilized. Each sample replicate was dried at 103°C for > 16 hours to determine samples dry weights. SigmaPlot 12 was used to graph data. Water content (WC) of each cultivar seed with and without the mucilage was calculated using the equation:

$$\text{Water Content (WC)} = \frac{\text{Fresh Weight (FW)} - \text{Dry weight (DW)}}{\text{Dry Weight (DW)}}$$

Estimating the osmotic pressure of sweet basil seed mucilage

Germination of 'Italian Large Leaf' sweet basil seeds was tested by incubating seeds in the dark in $11 \times 11 \times 4$ cm transparent covered plastic boxes (with feet removed) in a one-dimensional thermo-gradient table, on top of two thicknesses of germination blotter paper, the blotters were

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saturated with a 15 ml osmotic solution of PEG (polyethylene glycol 8000 molecular weight). PEG was used because it is a nonpenetrating, nonphytotoxic osmoticum. Seeds were hydrated in PEG solutions of two different water potentials: – 0.25 MPa, and PEG – 0.5 MPa, verified by osmometry (5500 Vapor Pressure Osmometer Wescor) at an experimental temperature of 25°C. The osmometer was calibrated with solutions of 1000, 290, and 100 mosmols/Kg to increase the accuracy when measuring samples with high water potential. Differences in germination performance and mucilage formation were observed as seeds were hydrated in each osmotic solution. Osmotic potential of sweet basil seed mucilage ('Italian Large Leaf', and 'Aroma 2') was measured after excision from fully hydrated seeds by placing 1 ml samples in the deep sample dish and operating the osmometer in the manual cooling mode at extended equilibration times up to 1 hr. The osmolality readings from vapor pressure osmometer were converted to water potential (Ψ) using Van't Hoff equation (Milburn, 1979).

Seeds cling and media test

Propose of the seed cling test was to test whether the pericarp mucilage could help seeds stick to a surface. Twenty fully hydrated 'Italian Large Leaf' sweet basil seeds with and without mucilage were placed on a glass and the angle was adjusted until the seeds moved due to the pull of gravity. Experiments were conducted at 25°C, 39% RH.

Basil seeds with and without mucilage were germination tested on five diverse types of media (clay, loamy clay, perlite, vermiculite and sand). The ability of seeds to penetrate these different types of media using different amounts of moisture was also tested. Each type of media was placed in cell packs (3×3 cm cell) and covered with a translucent tray dome to prevent desiccation. Three replications of total 10 'Italian Large Leaf' sweet basil seeds each were tested in each cell hydrated with 1, 2, 3, 4, 5 or 6 ml of distilled water pipetted on to the media in each cell. Following hydration the moist media and seeds were covered with a transparent ridged plastic dome, and incubated at room temperature at 25°C for 10 days. Germination and survival percentages were recorded daily throughout the testing period.

Results and discussion

Images of the dry seed and fully hydrated basil seed showed that a large volume of mucilage produced during imbibition (Fig. 2). The hydrated seed occupied more than 5 times greater volume than the dry seed (Fig. 1).

Mechanical removal of seed mucilage by rubbing seeds with sand paper or paper towels was effective but slow, tedious, and impractical for large quantities of seeds. The seeds of wet-seeded crops such as tomato are chemically cleaned to remove material tissues that adhere to the seed coat.

Other chemical methods is used to clean tomato seeds were investigated. Tomato seeds typically have mucilage around them during development that may be chemically removed using sodium hydroxide or sodium carbonate. For small quantities of seeds in cooler temperate areas, sodium carbonate is sometimes used to separate the tomato seeds from their mucilage. In this experiment basil seeds were treated with a 10% solution of sodium carbonate (washing soda). The seeds were soaked in the sodium carbonate solution for up to two days at room temperature, rinsed with water through a sieve, and subsequently dried. However, treatment with sodium bicarbonate did not effectively remove mucilage from basil seeds.

Another method is use diluted hydrochloric acid (HCl), often favored by large commercial producers as it produces a very bright clean seed sample. The actual dilution rate and duration of treatment used by the major commercial seed producers is usually a closely guarded secret. Producers of relatively small quantities of tomato seed find that 567 ml of concentrated hydrochloric acid stirred into 10 liters of seed mixture and left for half an hour is successful. It is very important that the acid is added to the seed mixture, and not the seed mixture to the acid, otherwise a dangerous effervescence will occur. People handling the concentrated and diluted acid solutions must wear appropriate face shields and protective clothing.

Diluted hydrochloric acid (HCl), is often favored by large commercial seed producers as it produces a very bright clean seed without pubescence or mucilage. The actual dilution rate and duration of treatment used by the major commercial seed producers is usually a closely guarded secret. Producers of relatively small quantities of tomato seed find that 567 ml of concentrated hydrochloric acid stirred into 10 liters of seed mixture and left for half an hour is successful. In this study, a diluted hydrochloric acid (HCl) (1:2 volume ratios of concentrated hydrochloric acid and distilled water) treatment effectively removed the mucilage from for three cultivars of sweet basil ('Italian Large Leaf', 'Genovese' and 'Aroma 2') seeds. This treatment was used to remove mucilage in all subsequent experiments unless stated otherwise.

There were no significant differences between the intact seeds and seeds without mucilage of each cultivar according to paired T-test (Sigma Analysis, both for germination percentage and mean time to germination (MTG, Table 1). The germination percentage was unchanged by moving the mucilage layer while the MTG increased slightly. This suggests that the mucilage did not affect seed viability and that seeds germinated more slowly without mucilage. The mucilage may aid in the hydration of the seeds by increasing contact with the media and increasing hydraulic conductivity between the seed and the surrounding environment. Another possibility is that the acid treatment may have damaged the seeds in a way that did not reduce viability but reduced the seed of germination. Since seed viability was not affected by the acid treatment, this technique was used to remove the mucilage layer so its effects on germination performance could be assessed. This was similar with other studies, showing the seeds contain mucilage in their pericarp had a higher germination rate than that of demucilaged seeds (Yang et al., 2010).

There were no significant differences in germination percentage or the MTG between the intact seeds and seeds without mucilage for each cultivar compared statistically using a paired T-test (Table 1). The germination percentage was unchanged by the removing the mucilage layer while the MTG increased slightly. This suggests that the mucilage did not affect seed viability and that seeds germinated more slowly without mucilage. The mucilage may aid in the hydration of the seeds by increasing contact with the media and increasing hydraulic conductivity between the seed and the surrounding environment. Another possibility is that the acid treatment may have damaged the seeds in a way that did not reduce viability but reduced the speed of germination. Since seed viability was not affected by the acid treatment, this technique was used to remove the mucilage layer so its effects on germination performance could be assessed. This was similar with other studies, showing the seeds contain mucilage in their pericarp had a higher germination rate than that of demucilaged seeds (Yang et al., 2010).

Sweet basil seed coat mucilage expands rapidly as it hydrates. When imbibed with water, the seed began producing mucilage within seconds. Within one minute, the seed was visibly swollen and covered with a thick layer of mucilage (Fig. 3A). This rapid expansion continued until the seed was fully hydrated after several minutes (Fig. 3B). The mucilage was tethered to the pericarp in long striated strands that were possibly composed of polysaccharide based on histochemical staining and other studies (Western, 2012; Razavi, 2009). The outer edge of the mucilage had coiled strands. Almost 90% of the mucilage was produced within first 20 minutes

(Fig. 3C). The mucilage expanded slowly after 20 minutes but continued to expand at a slower rate until expansion stopped after an hour (Fig. 3D). The staining patterns have different color shades, suggesting that the mucilage may have been composed of several different types of polysaccharides (Fig 3).

Basil belongs to the family Lamiaceae, and the production of mucilage in Lamiaceae is fruit mucilage also called myxocarpy (Western, 2012). The cross section light microscopy image of a basil seed showed the structure of the seed coat (Fig. 4). Outside of the seed coat is a layer of pericarp, and the mucilage layer was expanded from the pericarp tissue. Some studies of other mucilage producing seeds from Lamiaceae family such as Chia (*Salvia hispanica*) also showed similar structure like basil (study from Watchareewan Jamboonsri, a graduate student from University of Kentucky, unpublished data, not listed in the reference).

ESEM (Environmental Scanning Electronic Microscopy) images showed the dry intact seed (Fig. 5) had more fractured cells on outer pericarp surface where the mucilage was likely produced. Images of dry seed, treated with HCl to remove the mucilage-producing layer, had a smoother surface compared to untreated seeds capable of producing mucilage (Fig. 6). Images of dead seed that produced no mucilage were similar in appearance to seeds with the mucilage removed with acid treatment (Fig. 7). Dead seeds had a relatively smooth surface. The reasons why some seeds in the commercial seed lot were dead is unknown. Dead seeds appeared somewhat smaller, so one theory is that they were harvested at an immature stage of development and had not developed to the point where they were capable of producing mucilage. Some dead seeds in this study did produce small amounts of mucilage. Seeds that were microwaved prior to imbibition did not germinate but produced as much or more mucilage as fully viable live seeds. Therefore, mucilage itself is not required for germination as seeds with mucilage removed germinated normally and dead seeds also produced mucilage even when their embryos were nonviable. The clustered pieces material on the surface of dry intact fruit may be polysaccharides, which produced the mucilage, filled with coiled strands (Fig. 5). Some ESEM images of mucilage were taken after slowly increasing the relative humidity (RH) in the sample chamber containing the seed to 100% RH for 20 minutes in an attempt to visualize mucilage production at high resolution (Fig. 8). Unfortunately, this approach was unsuccessful because the electron beam interfered with visualization of mucilage production during the first few minutes of the process.

The seed hydration (Fig. 9) and dehydration (Fig. 10) time courses for seeds with and without the mucilage showed distinctive phases of hydration. The hydration curves of both intact seed cultivars were very similar bimodal curves with two distinct plateaus. The first increase occurred during the initial 15 minutes and corresponded with the hydration of the mucilage layer. The water content increased at a slower rate during the next 3 hours. The second increase in hydration started after the first plateau, and increasing slowly till the seeds were fully hydrated and reached a second plateau. This second increase and plateau corresponded to the hydration of the embryo. The seeds without mucilage had only one plateau for both cultivars, which reflected only the hydration of the testa and embryo (Fig. 9). So the bimodal hydration time course for the intact seeds from both cultivars was because of the mucilage formation, which caused the first rapid increase in water content while the second slower increase was due to embryo hydration. The dehydration curves illustrate very clearly that the mucilage does act as a water reservoir that allowed seeds to remain hydrated for several hours compared to seeds without mucilage that dried out more quickly (Fig. 10). This would give seeds in the wild a significant ecological advantage after a rainfall event. If the rain lasts longer than 30 minutes, the majority of mucilage could hydrate giving seeds more water content and greater volume.

Mucilage production has been proposed to play a role in germination. For example, the intact achenes of *Aytenisia sphaerocephala* (Asteraceae), which produce mucilage from their pericarp, had a higher germination percentage than demucilaged achenes (Yang et al., 2010). It has also been proposed mucilage producing seeds improve contact and adhesion to soil partials (Grubert, 1974). Other function of mucilaginous seed of *Salvia columbariae* (Limiaceae) was to collect sand so the seed would not be eaten (Western, 2012). Basil seed mucilage might have a similar ecological function.

The water content (dry weight basis) of the intact seeds for both 'Aroma 2' and 'Italian Large Leaf' sweet basil cultivars was almost four times greater than seeds without mucilage (Table. 2). That meant the mucilage held a significant quantity of water and acted as a reservoir for the seeds to store water, which could be used for seedling growth and development. Water potential measurements of the mucilage approached 0 MPa, suggesting that the water was loosely bound and readily available to drive expansive growth of the germinating seedling.

When seeds were hydrated in osmotic solutions of - 0.5 and - 0.25 MPa, there was greater inhibition of mucilage formation at the lower water potential. This illustrated the water potential threshold that seeds must obtain before mucilage formation is possible. Mucilage only forms when seeds are in contact with liquid water or at very high water potentials such as after a rainfall, consistent with the hypothesis that mucilage may serve as an anchor after significant rainfall events. This experiment also suggests that the water potential of the mucilage is approximately - 0.25 MPa because some mucilage production occurs at this level of hydration but complete production is still not possible. This observation is consistent with the direct measurements of mucilage water potential by osmometry, which for fully imbibed seeds was approximately - 0.2 MPa. The accuracy of water potential measurements decreases as one approaches 0 MPa because of the limitations of the isopiestic psychometric technique. Suffice to say, mucilage formation is only possible in well hydrated environments as seeds approach zero water potential. Consistent with other studies the osmotic solutions used for imbibition also slowed the rate of basil seed germination (Fig. 11).

Hydrated seeds with mucilage tightly adhere to a glass incline board even at 90° angle, and stayed even after 24 hours. Seeds without mucilage had much less adhesion and started to slip at a 20° angle after 15 minutes, and all seeds without mucilage lost adhesion and slid down the glass pane after 20 minutes. This experiment showed that basil seed mucilage can anchor seeds. This trait may help seeds from being moved in aqueous environments and allowing them to remain in locations more favorable for germination and establishment.

In the growing media test with limited seed sample size, the germination percentages of seeds with mucilage were higher than the demucilaged seeds on all types of media. The survival percentage of germinated seedlings after 10 days was also much higher than seeds without mucilage (Table. 4). The mucilage did not help radicles penetrate the soil of any type of medium. This suggests that the mucilage provided seeds with a reservoir of water that could be drawn upon to aid in survival during the initial stages of seedling growth. The test also showed that the basil seeds with mucilage have higher germination percentage not only on optimal germination tests under laboratory conditions but also in diverse soils and growing media. On sand and perlite, both germination percentage and 10 days survival percentages were much higher compare to seeds without mucilage although the experiment should be repeated with larger seed sample sizes before lasting conclusions can be formed. The survival percentages on sand and other

relatively types of dry media suggest that the mucilage may help seeds germinate, and seedlings survive.

Conclusion

The basil mucilage expands from the outer layer of the fruit pericarp and therefore it is not part of the seed *per se*. The mucilage expands very rapidly in water especially during the first 20 minutes of hydration. Intact basil seeds had almost 4 times greater water content than the seeds without mucilage. The mucilage is hydrophilic and the water potential of fully hydrated seeds approached zero water potential. This suggests that the mucilage acts as a reservoir to store loosely bound water after hydration that can drive expansive growth of young seedlings in dry environments and increase seedling survival percentages. A secondary function may be to anchor seeds to a favorable location and prevent movement by flowing water.

Table. 1. Effect of mucilage removal using diluted HCl on germination of 'Genovese', 'Aroma 2' and 'Italian Large Leaf' sweet basil at 32°C in an incubator on germination blotter paper.

Genotype and treatment	Germination (%) †	MTG(days) ‡
'Genovese'		
intact	87 ± 1.5	1.20 ± 0.08
w/o mucilage	88 ± 2.0	1.54 ± 0.07
'Aroma 2'		
intact	95 ± 1.0	1.22 ± 0.05
w/o mucilage	93 ± 1.5	1.65 ± 0.08
'Italian Large Leaf'		
intact	96 ± 1.4	1.49 ± 0.11
w/o mucilage	94 ± 1.0	1.60 ± 0.11
	NS	NS

†Means of four replications of 25 seeds each for intact type, and three replications of 50 seeds each for w/o (without) mucilaged seeds are shown ± SE

‡Mean time to germination: $\sum (N_iT_i)/\sum (N_i)$, where N_i is the number of new germinated seeds at time Ti after imbibitions calculated from four replications of 25 seeds each for intact seeds, and three replications of 50 seeds each for demucilaged seeds are shown ± SE.

NS, ** Nonsignificant or significant according to paired T-test.

Table. 2. Effect of seed coat mucilage removal on water content (dry weight basis) of 'Aroma 2' and 'Italian Large Leaf' sweet basil at 22°C, 35% RH.

Water Content (dry weight)	intact	w/o mucilage
'Italian Large Leaf'	4.71 ± 0.43	1.28 ± 0.03
'Aroma 2'	4.35 ± 0.03	1.01± 0.02

Table. 3. Water potential of the basil seed mucilage at 25°C.

Ψ (MPa)	20 min	40 min	120 min	150 min
'Italian Large Leaf'	- 0.076	- 0.088	- 0.106	- 0.155
'Aroma 2'	- 0.080	- 0.094	- 0.104	- 0.155

Table. 4. Germination / survival percentage of intact seeds and seeds w/o (without) mucilage germinated on different types of growing media after 10 days at 25°C (3 replications of 10 seeds total).

Genotype and treatment		Germination	/	Survival	(%)	
Water (ml)	1	2	3	4	5	6
Clay						
intact	0 / 0	0 / 0	0 / 0	40 / 20	50 / 30	80 / 60
w/o mucilage	0 / 0	0 / 0	0 / 0	60 / 40	60 / 60	60 / 60
Loamy clay						
intact	0 / 0	0 / 0	0 / 0	10 / 0	70 / 50	90 / 60
w/o mucilage	0 / 0	0 / 0	0 / 0	0 / 0	70 / 50	90 / 60
Vermiculite						
intact	30 / 10	90 / 50	90 / 50	100 /60	100 / 80	100 / 80
w/o mucilage	30 / 0	90 / 80	90 / 70	80 / 60	80 / 70	100 / 80
Sand						
intact	60 / 0	70 / 60	100 / 70	100 / 70	100 / 70	100 / 70
w/o mucilage	0 / 0	60 / 0	80 / 40	70 / 50	80 / 60	80 / 60
Perlite						
intact	80 / 0	90 / 0	90 / 50	80 / 50	90 / 50	80 / 40
w/o mucilage	40 / 0	50 / 0	50 / 10	70 / 40	70 / 40	60/ 30

Fig. 1. Images of intact dry basil seed (A) and fully hydrated seed (B) with mucilage (60×
magnifications).
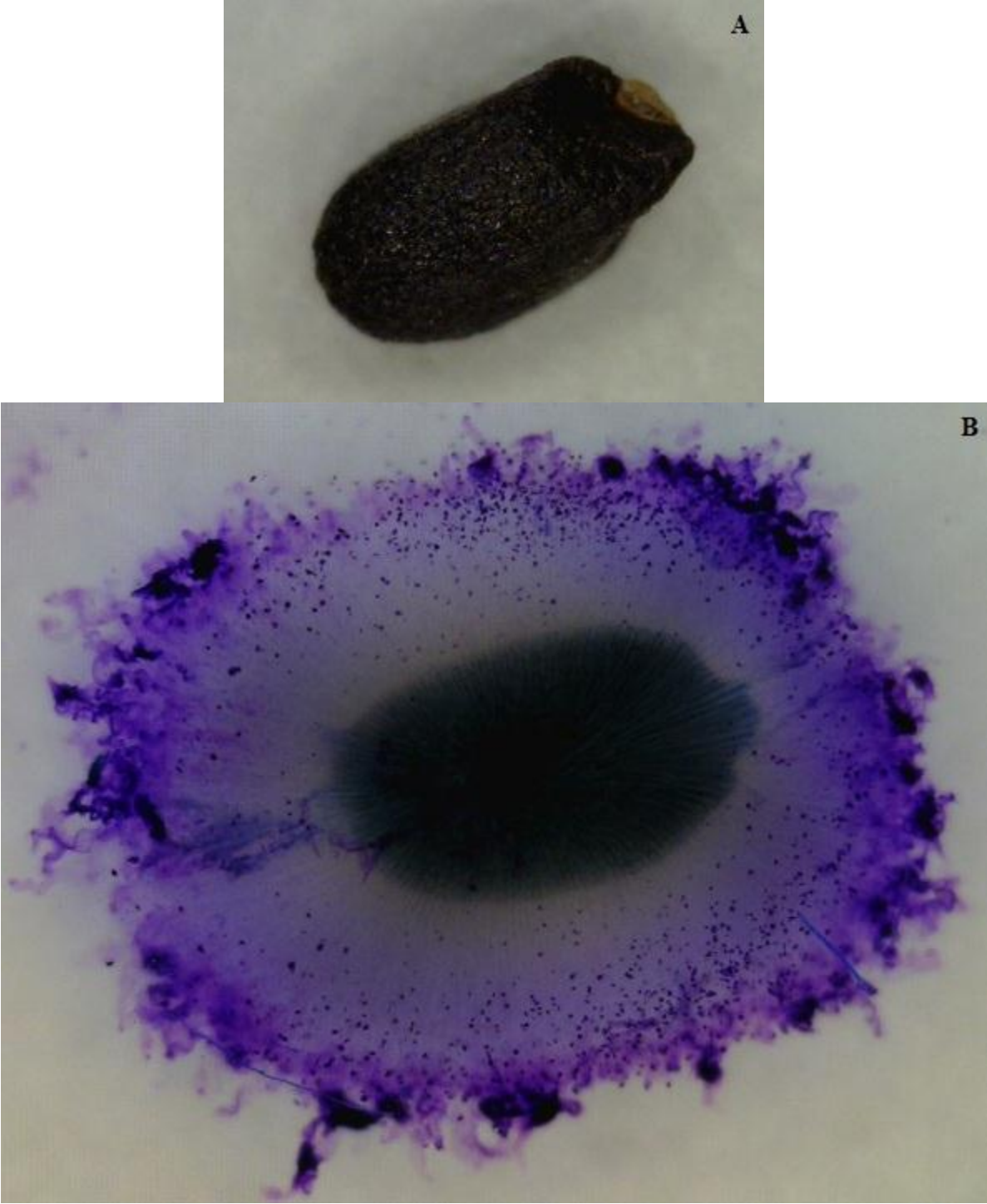
Fig. 2. Demucilaged basil seeds after HCl treated (60× magnifications). A, dry seed; B, hydrated seed.
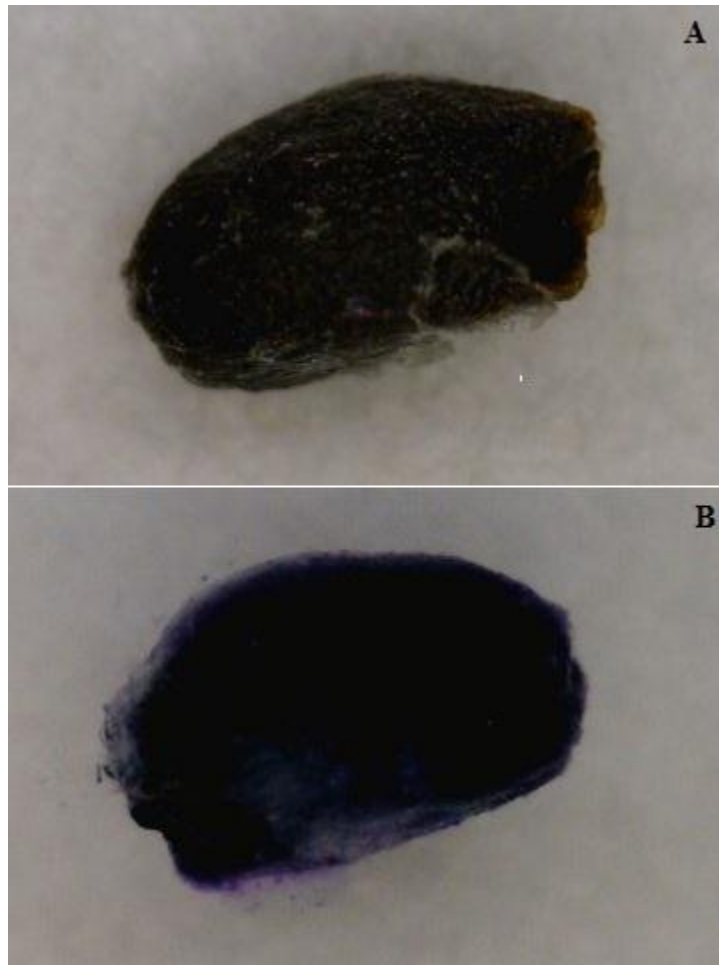
Fig. 3. The basil seed coat mucilage after imbibition in 0.1% Toluidine blue solution. (1) one minute; (2) 5 minutes; (3) 20 minutes; (4) 1 hour after imbibition. (60× magnifications).
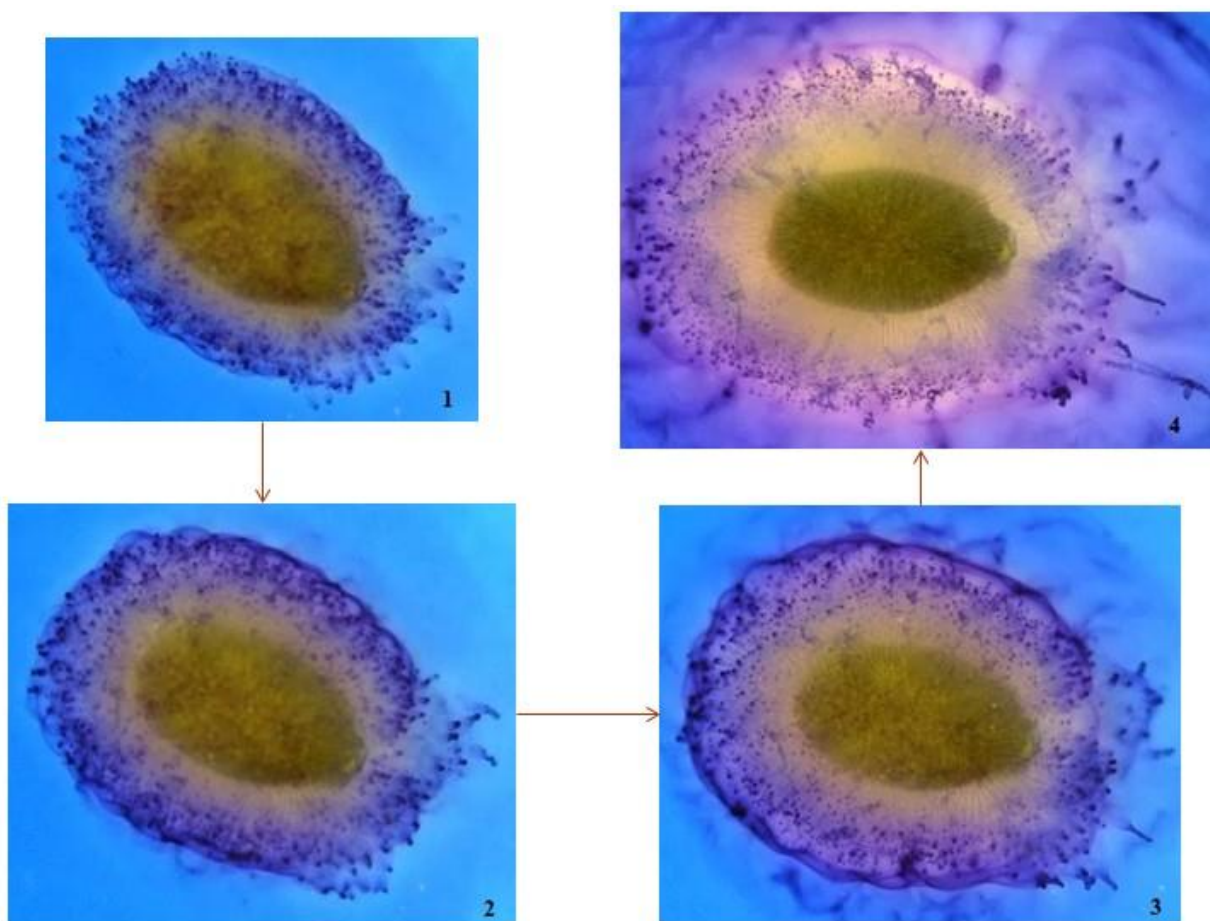
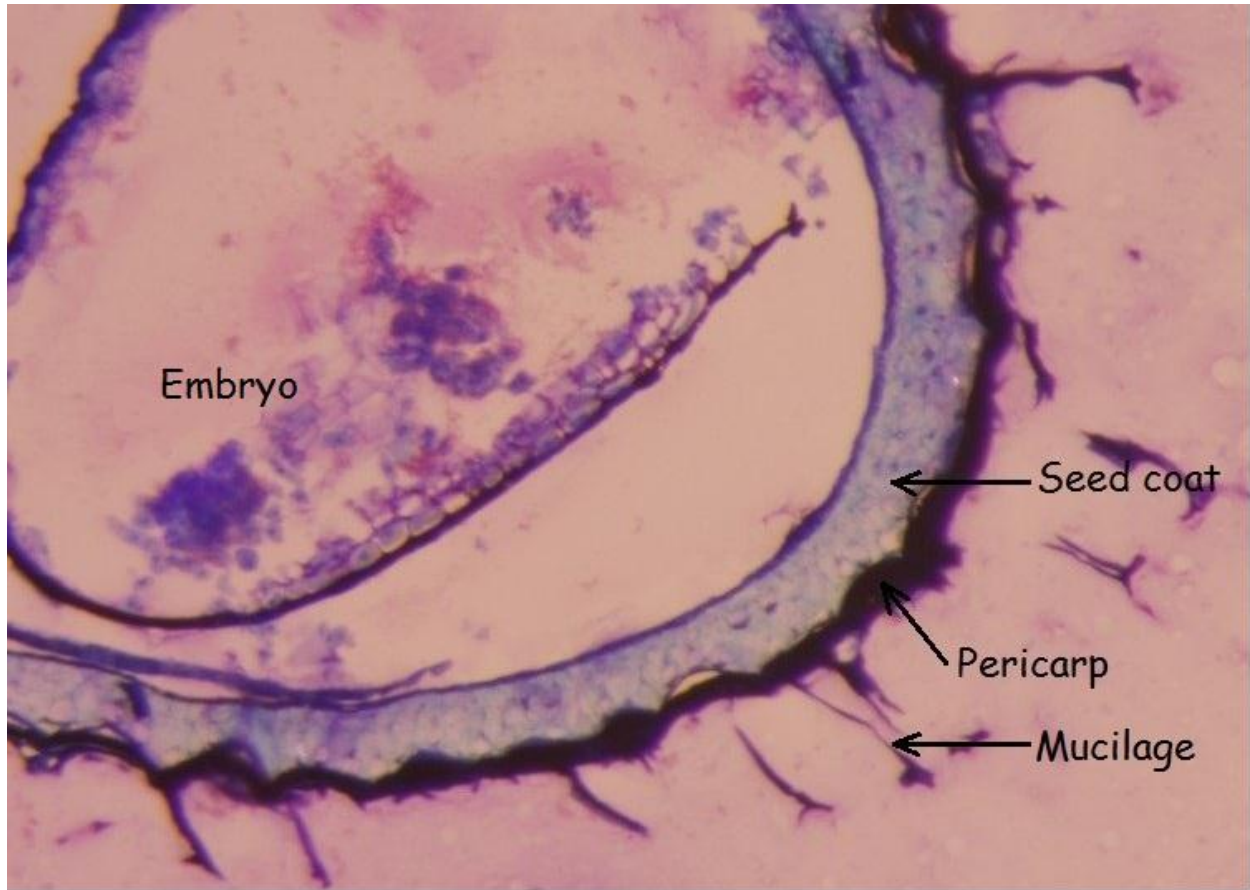Fig. 4. Light microscopy of basil seed cross section (400× magnifications).

Fig. 5. Dry intact basil seed images sputter coated prior to viewing by ESEM (Environmental Scanning Electronic Microscopy).
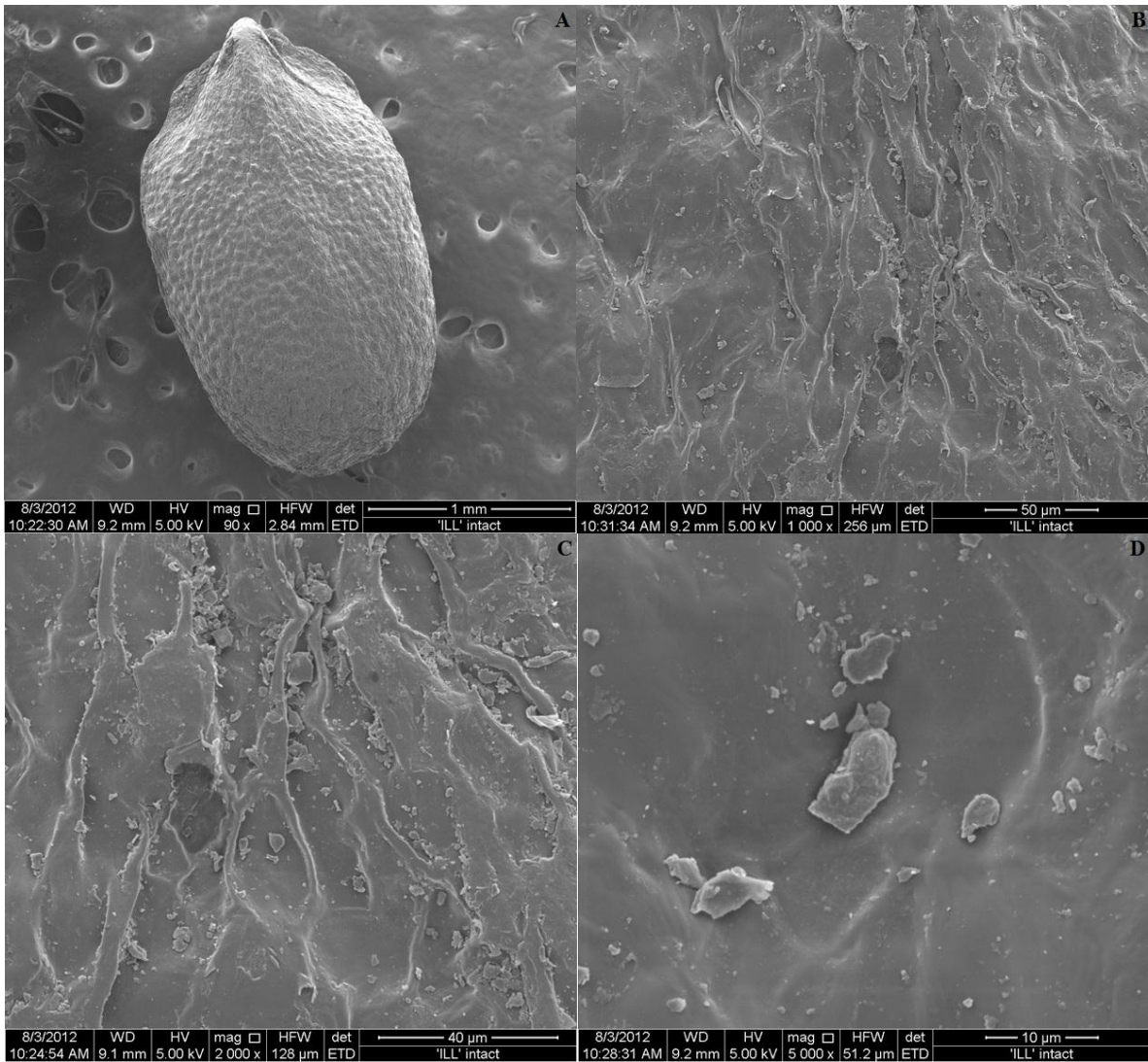
Fig. 6. Dry demucilaged basil seed images sputter coated prior to viewing by ESEM.
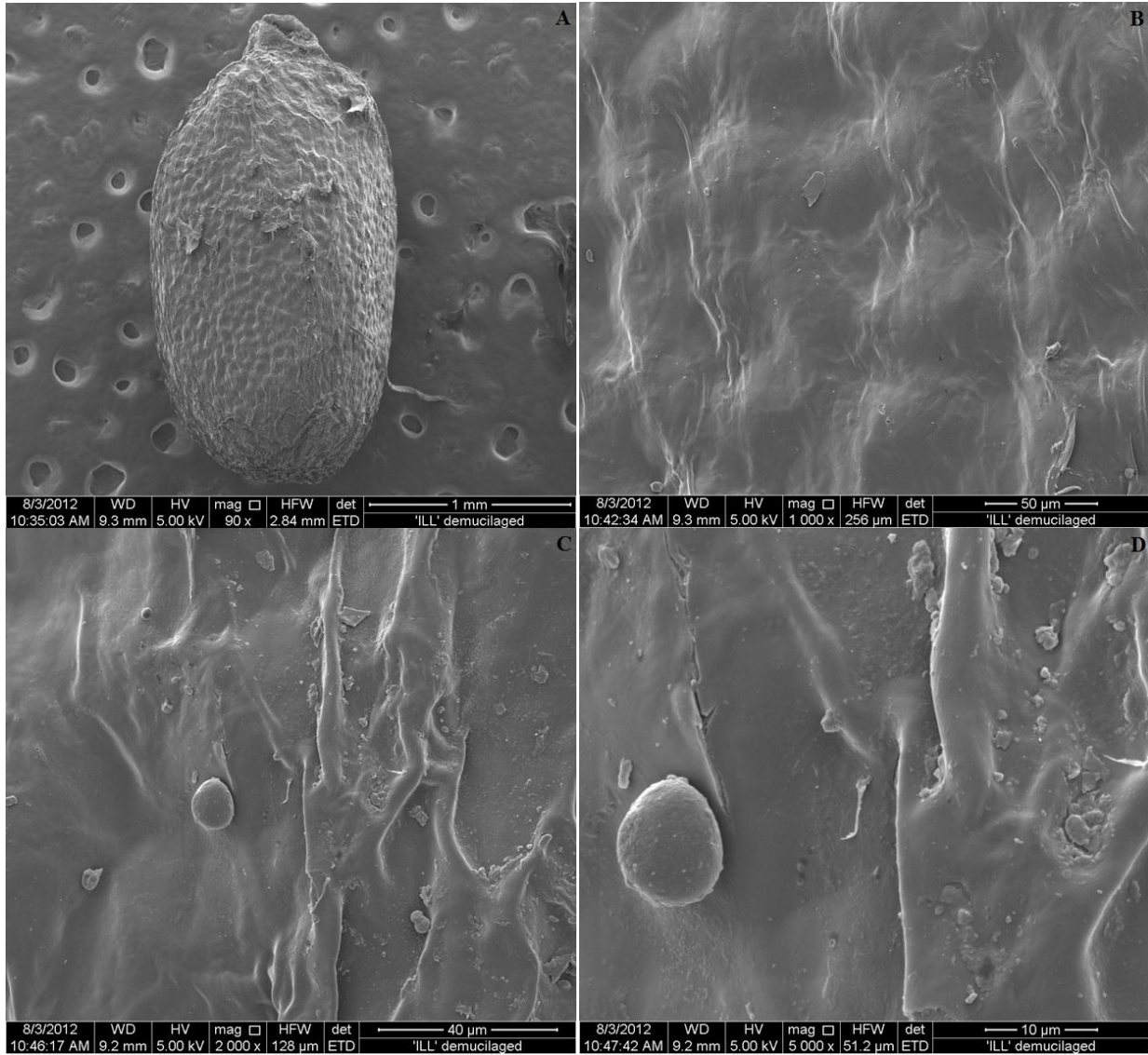
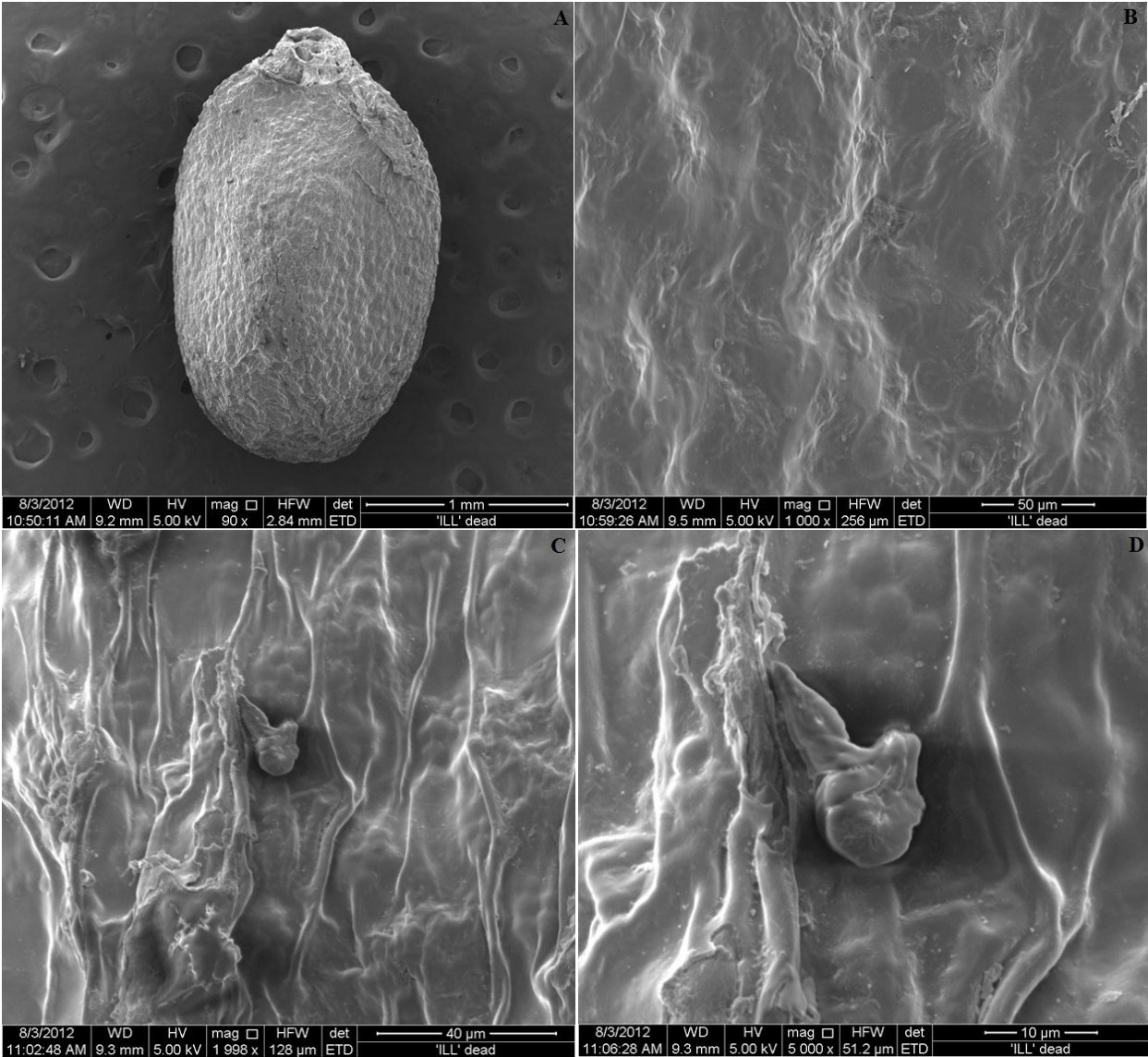Fig. 7. Dry dead basil seed images sputter coated prior to viewing by ESEM.

Fig. 8. Basil seed mucilage images by ESEM. All images were taken after increasing the relative humidity (RH) of seed to 100% RH for 20 minutes.
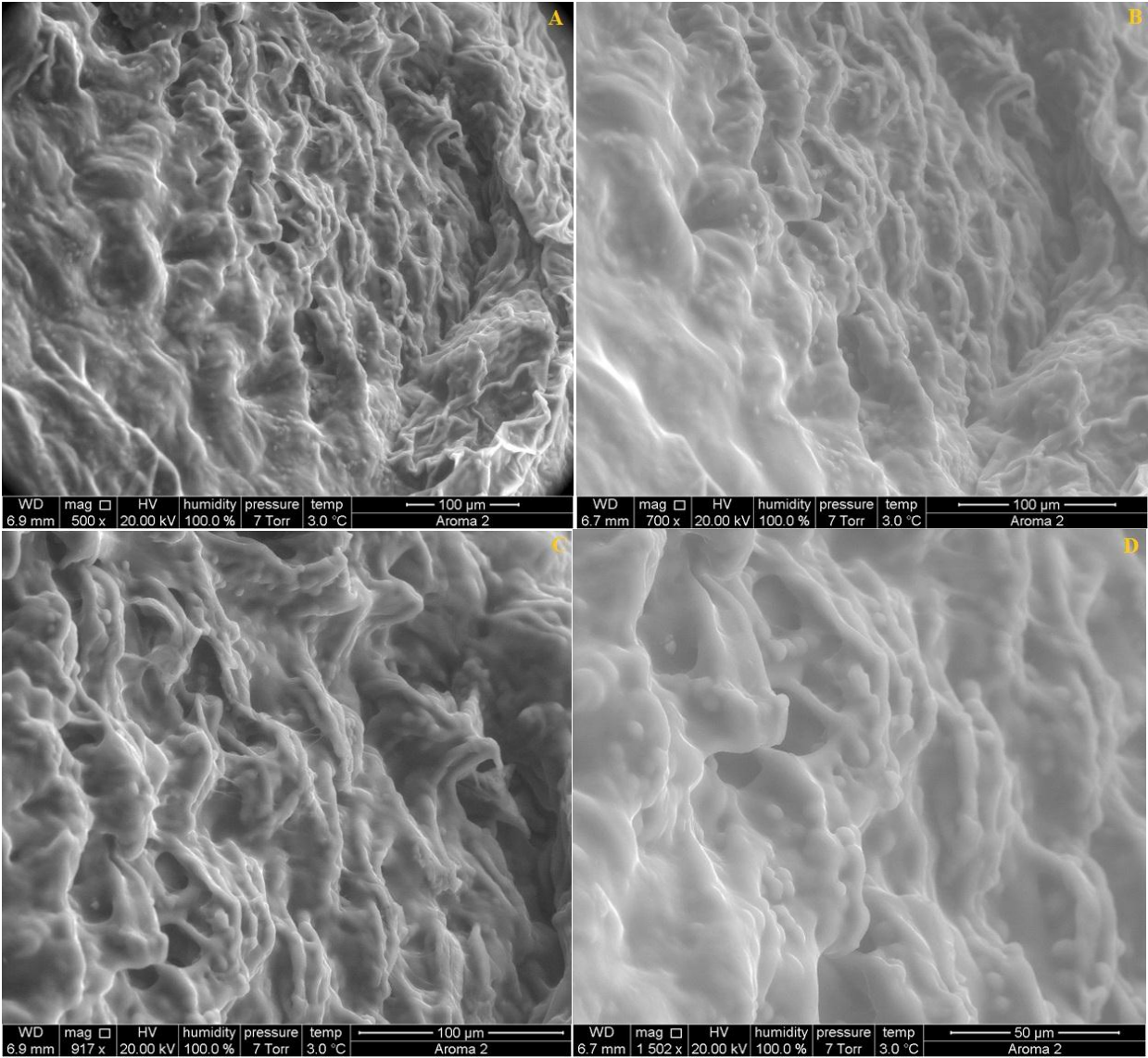
Fig. 9. Effect of seed coat mucilage removal on weight of 'Aroma 2' and 'Italian Large Leaf' sweet basil during seed hydration at 22°C.
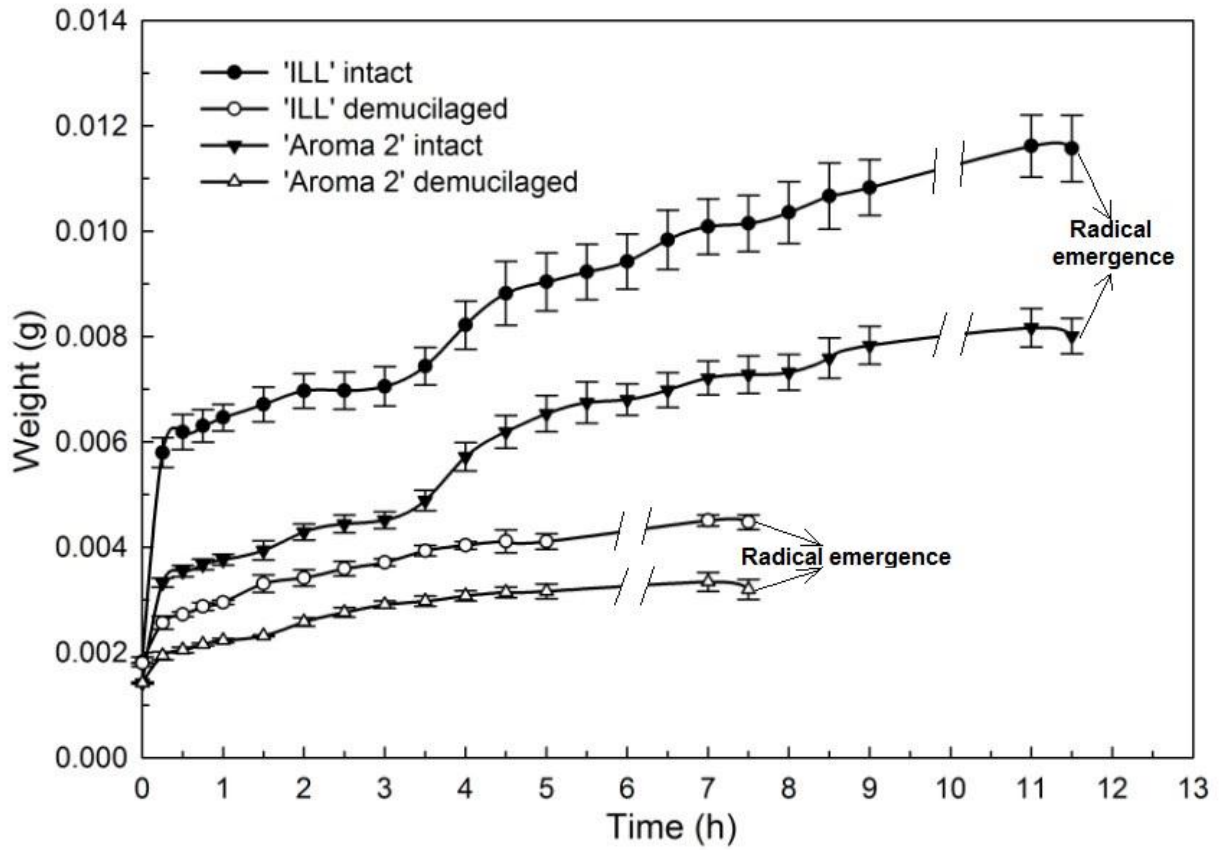
Fig. 10. Effect of seed coat mucilage removal on weight of 'Aroma 2' and 'Italian Large Leaf' sweet basil cultivars during seed dehydration at 22°C.
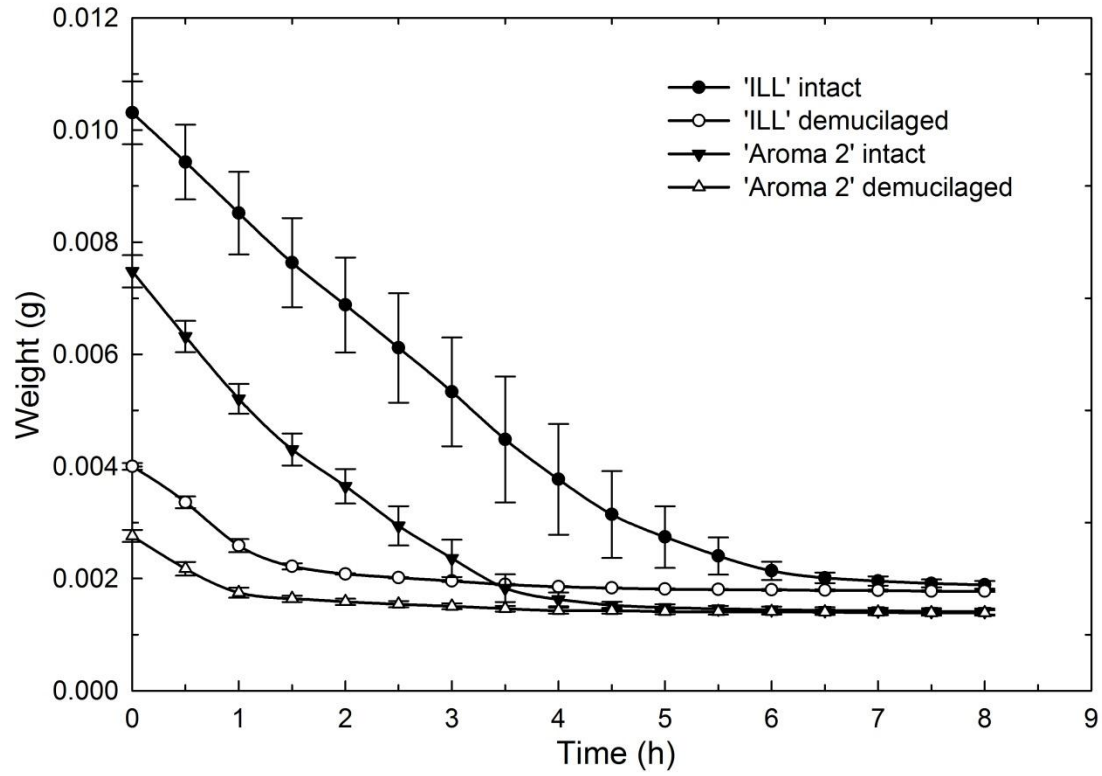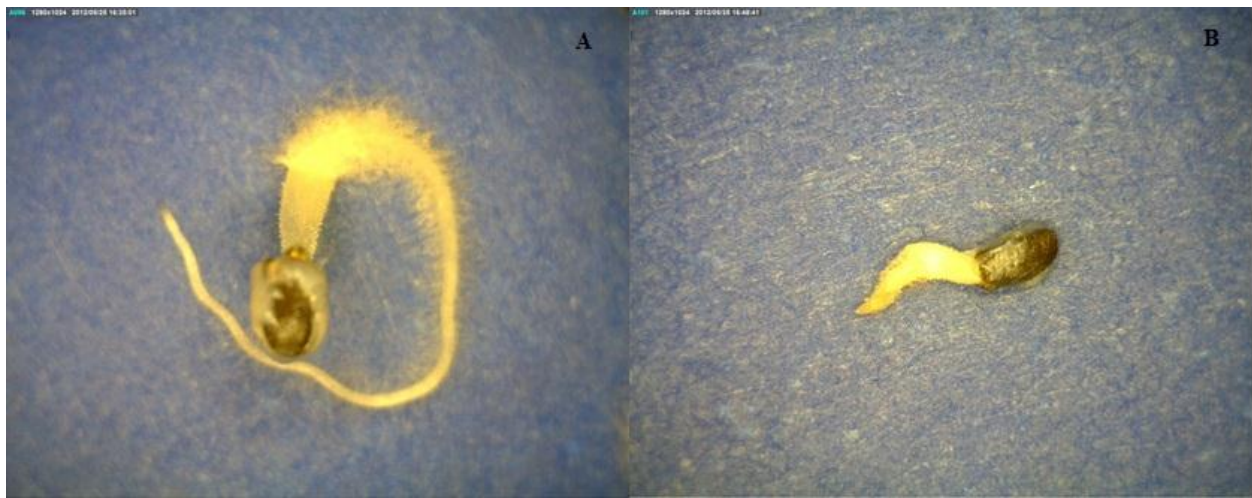
Fig. 11. Basil germination after 4 days imbibition on PEG Solutions (A, PEG - 0.25MPa, 25°C; B, PEG - 0.5MPa, 25°C).

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