

## Seed Development and Maturation of Eryngo (*Eryngium foetidum* L.)

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### ABSTRACT

Flowering pattern and seed development were studied in two eryngo accessions (EF006 and EF007) to determine the optimum time of harvest for seed production. Flowering peak was 117 days after transplanting (35.6 umbel heads) and 121 days after transplanting (38.7 umbel heads) in EF006 and EF007 accessions, respectively. Seed in both accessions reached their physiological maturity 40 days after anthesis, with 93.75-95.75% germination when the seed head had just begun to turn brownish black in color. Shattering, however, commenced 65 days after anthesis.

**Key word:** seed development, optimum harvest time, seed germination

### INTRODUCTION

Seed crops must be harvested when seed quality is at maximum. Some physiological markers have been used in order to identify the time of maximum seed quality. Harrington (1972) proposed the hypothesis that maximum seed quality was achieved when seeds reached maximum dry weight at the end of the seed filling period. This hypothesis has been supported by results in *Zinnia violacea* Cav. (Miyajima, 1997), *Brassica napus* L. (Still and Bradford, 1998) and *Daucus carota* L. (Nascimento *et al.*, 2003).

Seed maturity effects on seed quality are particularly evident in indeterminate crops. For example, flowering in carrots progresses from the 1<sup>st</sup> umbel to the 2<sup>nd</sup> and 3<sup>rd</sup> umbels during reproductive development (Oliva *et al.*, 1988). In these cases, the effects of maturity on seed quality are exacerbated by the fact that these crops exhibit shattering (or shedding) of seeds as they mature. Thus, delaying harvest to allow later developing seeds to mature risks losing mature seeds to

shattering. On the other hand, early harvesting results in more poor quality immature seeds that may be difficult to remove by standard cleaning and grading techniques (Bradford, 2004).

Eryngo (*Eryngium foetidum* L.) is known to be a native of Central and Latin America, from southern Mexico to Panama through Brazil and from Cuba to Trinidad. It was introduced into South-East Asia by the Chinese as a substitute for coriander. It is an aromatic plant which is usually grown as a leafy vegetable used as a seasoning and for medicinal purpose in various countries such as Vietnam, India and in the Amazon region (De Guzmao *et al.*, 2002).

The effect of umbel head maturity at harvest on seed quality has not been reported for eryngo. This information is needed to determine the stage of harvest attaining maximum germination and vigor. Therefore, the objective of this work was to determine the physiological changes during seed development and correlated them with visual indicators to determine optimum harvest time.

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## MATERIALS AND METHODS

### Study sites

The experiment was conducted at the Tropical Vegetable Research Center (TVRC), Kasetsart University, Khampaeng Saen Campus, Nakhon Pathom Province, Thailand, from August 2003 to March 2004.

### Plant materials

Seeds of two eryngo accessions (EF006 and EF007) maintained at TVRC were sown in a peat medium in plastic trays containing 104 inverted cone cells with a depth of 5.7cm and a volume of 20 cm<sup>3</sup>. Plants were thinned to one per cell 4 weeks after seeding and transplanted to 6-inch round pots in a rain shelter 49 days after seeding. During flowering period, from August 2003 to March 2004, all heads of the 3<sup>rd</sup> umbel order were tagged individually and the ages of seeds monitored and expressed as days after anthesis. Seeds from 5 to 70 days after anthesis (at intervals of 5 days) were collected and analyzed.

### Flowering pattern

Each week, newly-opened flowers of each accession were labeled with different colored nylon yarn. The days to reach anthesis for the first umbel to the tenth umbel, numbers flower/plant/week (from 20 plants) and numbers of seed/umbel head (from 20 tertiary umbel heads) were recorded. Thereafter, days to peak flowering were determined for both accessions.

### Seed weight and moisture content

Seed moisture content was measured by the hot-air oven method (ISTA, 2003). Pre-weighed fresh seeds were dried in an oven at 103° C for 16 hours, and dry weight was recorded. Seed moisture content was calculated using the following formula:

$$\% \text{ moisture content (w.b.)} =$$

$$\frac{\text{Fresh weight of seed} - \text{Dry weight of seed}}{\text{Fresh weight of seed}} \times 100$$

### Germination (GERM) and germination index (GI)

The harvested seeds were transferred to a nylon net bag and dried in ambient conditions for 5 days. Seeds were then placed in a controlled room (20°C, 30% relative humidity) to minimize the loss of seed moisture content (SMC). When the seed lot weight remained constant (around 7% SMC), a germination test (four 100 seed samples) was carried out on two layers of germination paper in rectangular transparent plastic boxes (14 × 9 × 5 mm) incubated at 20-30°C (16 hours and 8 hours, respectively). Normal seedlings were evaluated daily as described by International Seed Testing Association (ISTA, 2003) and germination index (GI) was calculated by the following formula as proposed by AOSA (1983).

$$GI = (N_i / D_i)$$

Where  $N_i$  = number of normal seedlings counted at  $i^{\text{th}}$  date.

$D_i$  = Number of days required to the  $i^{\text{th}}$  germination.

## RESULTS AND DISCUSSION

### Flowering pattern

Eryngo flowers in a compound inflorescence starting from the first umbel to the second umbel and so on during reproductive development. Within each umbel head, the flowers open from the outside ring of florets to the central ring which is a characteristic of the Umbelliferae plants.

Anthesis of the 1<sup>st</sup> umbel head occurred 73 and 77 days after transplanting in EF006 and EF007, respectively. The peak flowering was reached at 117 days after transplanting in EF006 and 121 days after transplanting in EF007 (Table 1). The flowering interval between the 1<sup>st</sup> and the 2<sup>nd</sup> umbels was about 5 days, and up to 7-8 days between the 9<sup>th</sup> and the 10<sup>th</sup> umbel orders

(Table 2). The numbers of flowering umbel head at differing umbel orders fitted the quadratic curves as indicated by the high coefficients of determination ( $r^2$ ). According to these regression equations, the number of umbel heads rapidly increased up to 35.2 and 38.7 heads at the eighth umbel order in EF006 and EF007 accessions, respectively, and then there was a sharp decline in higher umbel orders (Figure 1). Thus, both accessions had a similar flowering pattern with only one peak.

Because both accessions had only one peak flowering, determination of optimum harvest time was relatively easy. However, EF007 accession had more umbel heads at peak flowering and seeds per umbel than EF006 accession. Therefore, for seed production at TVRC, it was possible that EF007 accession could have a higher yield than EF006, due to the fact that plants with high flower usually being correlated with high seed yield (Soffer and Smith, 1974).

**Table 1** Days to peak flowering, number of umbel heads at peak flowering and number of seeds per head at the 3<sup>rd</sup> umbel order.

Accessions flowering <sup>1/</sup>	Days to peak at flowering peak	Number of heads/plant	seeds/head <sup>2/</sup>
EF006	117	35.2	60.0
EF007	121	38.7	73.2

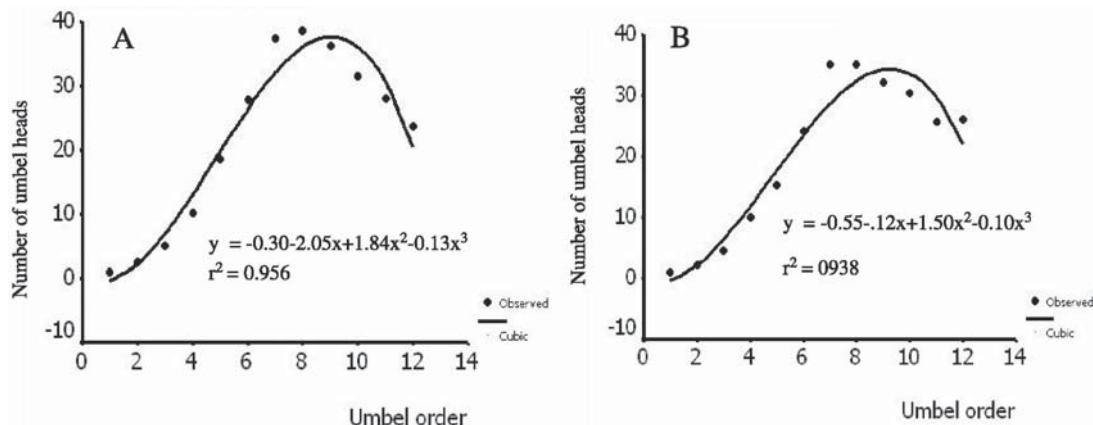
<sup>1/</sup> Days after transplanting

<sup>2/</sup> Obtained from 3<sup>rd</sup> umbel order

**Table 2** Days to anthesis<sup>1/</sup> at different umbel orders after transplanting.

Accessions	Umbel orders									
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>
EF006	73	78	84	91	98	104	111	118	125	133
EF007	77	82	88	95	102	108	115	121	127	134

<sup>1/</sup> (50% of floret blooming)



**Figure 1** Relationship between number of umbel heads and umbel order of EF006 (A) and EF007 (B). Coefficients of determination ( $r^2$ ) are significant at  $P \leq 0.01$ .

### Seed weight and moisture content

The fresh weight of eryngo seeds differed significantly among the collection periods (Figure 2). Initially, seed fresh weight increased from 0.55 g in EF007 and 0.62 g in EF006 10 days after anthesis to a maximum of 0.66 g 20 days and 25 days after anthesis, respectively. Seed dry weight increased from 0.10 g in EF007 and 0.12 g in EF006 10 days to the respective maximum of 0.40 g and 0.41 g 40 days after anthesis. This indicated that seed of both accessions reached physiological maturity (PM) 40 days after anthesis. A similar pattern has been reported in other plant species e.g., *Hibiscus esculentus* L. (Demir, 1994), *Brassica napus* L. (Still and Bradford, 1998) and *Daucus carota* L. (Nascimento *et al.*, 2003). However, at physiological maturity seed moisture content was still too high (23.7-25.9%) for harvesting. Thus, harvest date should be later than physiological maturity but before seed shattering.

In the present study, it was found that seed shattering started 65 days after anthesis in both accessions. Therefore, there was at least a 25-day window (days from PM to shattering) which mature seed could be harvested before seeds

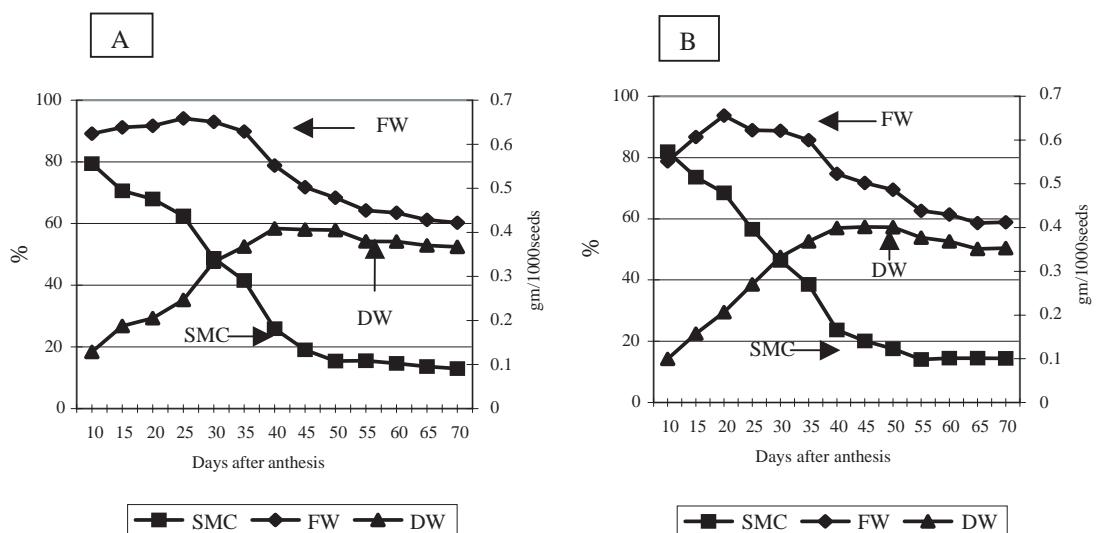
began to shatter.

### Seed germination and germination index

5 and 10 days after anthesis, the seed was still immature and showed no germination in both accessions. Seed started to germinate 15 days after anthesis or 25 days before it reached physiological maturity (Figure 3). Seeds harvested during 15-25 days after anthesis were able to germinate, but the germination was lower than 80%. Maximum germination was obtained 40 days after anthesis (95.75% in EF006 accession and 93.75% in EF007 accession). In the last harvest, however, germination was obtained at 90% in both accessions. Germination index followed the same trend as germination in both accessions (Figure 3).

### Changes in umbel head and seed color

Determination of seed age by days after anthesis alone is considered an inaccurate marker of seed development as the environment and micro-environment in which the plants are grown affect the rate of seed development and maturation (Yang *et al.*, 2004). Seed maturity must be related

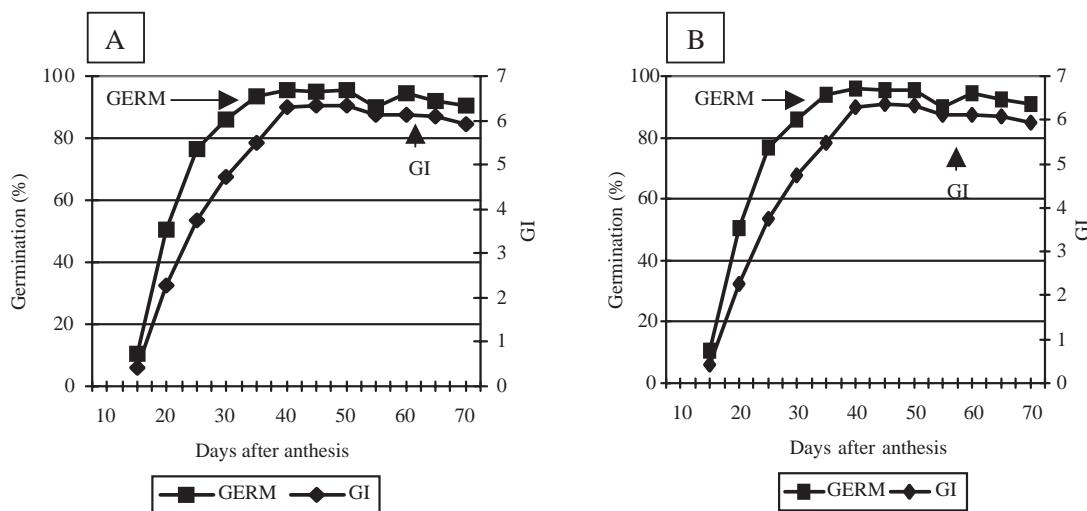


**Figure 2** Changes in fresh weight (FW), dry weight (DW) and seed moisture content (SMC) during seed development (A) EF006 and (B) EF007

to the color of fruit and seed coat, fresh and dry weight and seed moisture content (Thomsen, 2000).

Changes in color of umbel head and seed during their development are illustrated in Table 3. The umbel head exhibited a series of color changes from green 5-15 days after anthesis to yellowish brown 25-30 days after anthesis, and finally to brownish black at PM (40 DAA). Seed

color followed the same trend as umbel head color and was brownish black at PM. At this stage, both umbel head and seed color clearly indicated that seeds were mature. Therefore, it is useful to know the morphological and physical changes that occur during the maturation of seed, as such information may determine the proper time of seed harvest (Yang *et al.*, 2004). Similar color changes with maturity have been shown in many plants such as



**Figure 3** Changes in germination percentage (GERM) and germination index (GI) during seed development of (A) EF006 and (B) EF007

**Table 3** Changes in umbel head and seed color at different development stages of eryngo .

Days to anthesis	Umbel head color	Seed color
5	green	light green
10	green	light green
15	green	yellowish green
20	light green	greenish yellow
25	yellowish green	yellowish brown
30	yellowish brown	light brown
35	brown	brown
40	brownish black	brownish black
45	brownish black	brownish black
50	brownish black	brownish black
55	brownish black	brownish black
60	brownish black	brownish black
65	brownish black	brownish black
70	brownish black	brownish black

light brown seed integument in *Atriplex cordobensis* (Aiazzi *et al.*, 1998), brown seed in carrot (Rubatzky *et al.*, 1999) and yellow pod in common vetch (Samarah *et al.*, 2004). Therefore, the changes of umbel head and seed color would be a good indicator for predicting eryngo seed maturation.

Overall, the optimum time for harvesting should take into account. Firstly, days to peak flowering and secondly days from anthesis to PM. From the present work (conducted from August to March), the optimum times for seed harvest in EF006 and EF007 accessions were 157 and 161 days (days to peak flowering + days from anthesis to physiological maturity) after transplanting or 204 and 208 days after seeding, respectively. However, if transplanting date was not in the same period as the present experiment, harvest date might be different. Thus, seed color should be used to predict the optimum harvesting time for eryngo seed production.

## CONCLUSION

Eryngo reached peak flowering at the 8<sup>th</sup> umbel orders or 117 days after transplanting in EF006 accession (35.6 umbel heads) and 121 after transplanting in EF007 accession (38.7 umbel heads). Seed of two eryngo reached physiological maturity (PM) 40 days after anthesis. Seed began to germinate 15 days after anthesis and maximum when reached physiological maturity. Changes in umbel head and seed color from brown to brownish black clearly indicated that the physiological maturity and germination maturity were reached. The optimum times for seed harvest in EF006 and EF007 accessions were 157 days and 161 days after transplanting, respectively.

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