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# Changes in seed quality of dill (*Anethum graveolens*) ecotypes in response to water limitation and harvest time

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## Abstract

A split plot experiment based on randomized complete block design with three replications was carried out in 2015 to determine the best stage for harvest of three dill (*Anethum graveolens*) ecotypes (Varamin, Isfahan, Malayer) under different irrigation conditions (irrigation after 70, 100, 130 and 160 mm evaporation from class A pan), in order to produce high quality seeds. Results clearly showed that seed quality of all ecotypes under different irrigation intervals was increased with improving seed development on the mother plants. Maximum seed quality on the basis of electrical conductivity of seed leachates, germination percentage and rate and seedling dry weight was obtained at about 2-12 days after mass maturity, depending on ecotypes and irrigation intervals. Earlier harvests due to immaturity and later harvests because of aging reduced seed germination and vigor. Seed vigor of all dill ecotypes gradually diminished with declining water availability to the mother plants. It was, therefore, concluded that irrigation of dill plants particularly during seed filling could improve the quality of seeds. Nevertheless, the best seed moisture content for harvesting dill plants and producing high-quality seeds was about 15-20%. The most vigorous seeds on the basis of germination rate and seedling dry weight were produced by Varamin, followed by Malayer ecotypes.

**Keywords:** Dill; Germination; Irrigation interval; Maturity; Seed quality

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## Introduction

Dill (*Anethum graveolens*) is a plant from Apiaceae family, which can be used as medicine and vegetable (Hosseinzadeh *et al*. 2015). Seed production and quality of dill could be influenced by environmental stresses such as drought.

A vast part of Asia and beyond during the last decades are subjected to some levels of water stress, which considerably limits crop production in some countries. Water deficit or drought is known as an important limiting factor of growth and yield of aromatic and medicinal plants (Naeem *et al*. 2013). In such environments farmers require trusty and successful establishment of their crops. Seed quality is an important factor for sustainable and profitable production of crops, especially under unfavorable environmental conditions (Finch-Savage and Bassel 2016). Seed quality can influence crop yield via a direct effect on the rate of seedling emergence and an indirect effect on the percentage of field emergence (Ghassemi-Golezani 1992; Finch-Savage 1995).

Seeds on the mother plants progressively increase the ability of germination and vigor, which are the major aspects of physiological quality. Seeds may attain maximum quality at physiological maturity (PM) (Ghassemi-Golezani *et al.* 2011; Bewley *et al*. 2013). Seed quality may also continue to enhance after independence from mother plant and so PM can be achieved some times after mass maturity (MM; end of seed filling phase) and often before harvest maturity (HM) (Ghassemi-Golezani *et al.* 2015; Finch-Savage and Bassel 2016). Thereafter, seed quality begins to decline as seeds age and loses viability (Finch-Savage and Bassel 2016). However, the exact time of attaining maximum seed quality may differ among species (Tekrony and Egley 1997; Ghassemi-Golezani and Ghassemi 2013).

Commercial seed companies prefer to select locations with favorable climates to ensure production of high-quality seeds. It has been reported that the quality of seeds can be reduced by several factors such as drought, insects, pathogens, and very high and low temperatures during maturation and production stages (França-Neto *et al.* 2000). Hampton *et al.* (2013) found that high temperature stress both before and after PM decreases seed vigor. Production of seeds with low physiological quality due to water limitation was also reported for sesame (Silva *et al.* 2016). Similar results were observed by Ghassemi-Golezani and Ghassemi (2014) in chickpea. In contrast, Ghssemi-Golezani and Hosseinzadeh-Mahootchi (2009) in faba bean and Castaneda-Saucedo *et al.* (2009) in dry bean showed that seed quality was not significantly affected by water limitation.

Since, the effect of water stress on seed quality development of dill is not clear, this research was designed to investigate the changes in seed physiological quality of dill ecotypes under various irrigation intervals in order to determine the best stage of harvest for producing high quality seeds.

## Material and Methods

A split plot experiment based on randomized complete block design with three replications was carried out at the Research Field of University of Tabriz, Iran (latitude 38.050 N, longitude 46.170 E, altitude 1360 m above sea level) in 2015 to evaluate changes in seed physiological quality of dill ecotypes under well and limited irrigation conditions. The climate is characterized by mean annual precipitation of 313 mm, mean annual temperature of 10 ˚C, mean annual maximum temperature of 15.8 ˚C and mean annual minimum temperature of 2.51 ˚C. Factors were four irrigation intervals ($I_1$, $I_2$, $I_3$, $I_4$: irrigation after 70, 100, 130 and 160 mm evaporation from class A pan, respectively) which were arranged in main plots and three ecotypes (Varamin, Isfahan, Malayer) that were considered in sub-plots.

Each plot consisted of 6 rows of 4 m length, spaced 25 cm apart. The seeds were treated with 2 g/kg Benomyl before sowing. Then seeds were hand sown in about 2 cm depth of soil, spaced 5 cm apart. Seeding rate was 80 seeds/$m^2$. All plots were irrigated immediately after sowing. Subsequent irrigations were carried out on the basis of evaporation from class A pan. Hand weeding was done as required. After seedling emergence, plants per unit area were reduced to 56 plants/$m^2$ by thinning.

After seed formation, 38 plants from each plot were harvested at seven day intervals in seven stages. Then, seeds were detached from umbels and seed moisture content was determined in accordance with ISTA (2010) rules, using two replicates of 5 g seeds. Subsequently, seeds of 10 plants were dried in an oven at 130 ˚C for an hour and mean seed weight of each sample was determined. The remaining seeds of 28 plants from each plot were air dried at 18-20 ˚C. Seed samples were separately kept in sealed bags within refrigerator at 3-5 ˚C. Seed quality tests were carried out at Seed Technology Laboratory of the University of Tabriz, Iran.

Two replicates of 50 seeds from each sample were weighed ($SW_1$, $SW_2$) and then seeds of each replicate were immersed in 250 ml deionized water in a container at 20 °C for 24 hours. The seed-steep water was then gently decanted and EC ($EC_1$, $EC_2$) was measured using an EC meter. Then, mean conductivity per gram of seed weight for each sample was calculated (Powell *et al.* 1984):

$$EC\ (\mu s/cm/g) = [(EC_1/SW_1) + (EC_2/SW_2)]/2$$

Four replicates of 25 seeds from each sample were tested for germination between double layered moist rolled papers (ISTA 2010). The rolled papers with seeds were put into the plastic bags to avoid moisture loss and were kept in a refrigerator at 5 ˚C for seven days to eliminate probable dormancy. Seeds were allowed to germinate at 20 ± 1°C for 14 days. Germination was considered to have occurred when the radicles were 2 mm long. Germinated seeds were recorded every 24 h for 14 days. Rate of seed germination (R) was calculated according to Ellis and Roberts (1980):

$$GR = \sum n / \sum Dn$$

where, $n$ is the number of seeds germinated on day $D$, $D$ is the number of days from the beginning of the test and $GR$ is the mean germination rate. At the end of this test (14 days), percentage of germination was also determined.

At the end of germination test (14 days), radicles and shoots of normal seedlings were detached from the remaining seed reserves and dried in an oven at 80 ± 2 °C for 24 hours. The dried radicles and shoots were weighed to the nearest milligram and consequently mean seedling dry weight was determined.

Analysis of variance of the data appropriate to the experimental design was carried out, using MSTAT-C software. Means of the traits were compared by Duncan's multiple range test at $p \leq 0.05$. Excel software was used to fit regression lines on the mean data.

## Results

Seed weight of dill ecotypes was enhanced with increasing grain filling duration up to achieving maximum weight, and thereafter no changes were occurred. Mass maturity (end of seed filling) under $I_1$, $I_2$, $I_3$ and $I_4$ were achieved at 58, 60, 58 and 56 days after flowering for Varamin, at 59, 59, 56 and 55 days after flowering for Isfahan and at 60, 59, 56 and 55 days after flowering for Malayer ecotypes, respectively. In general, water deficit decreased maximum seed weight of dill ecotypes (Figure 1).

Electrical conductivity (EC) of seed leachates for all dill ecotypes under all irrigation intervals was very high at early stages of seed development, but it was diminished with progressing seed development. Minimum electrical conductivity for all ecotypes under different watering levels was achieved at 2-12 days after mass maturity.

Seed moisture content at this stage was about15-20%. EC of seed leachates at the latest harvest slightly increased. However, the minimum electrical conductivity of all ecotypes under severe water stress ($I_4$) was higher than the other irrigation treatments (Figure 2). Germination percentage of dill ecotypes increased with improving seed development.

Maximum seed germination percentage was occurred at 2-6 days after mass maturity, depending on water supply and ecotypes. Thereafter, germination percentage of seeds under various irrigation treatments decreased at later harvest. Maximum germination percentage of seeds for Varamin under different irrigation treatments was almost similar, but for Isfahan and Malayer under normal irrigation ($I_1$) and mild stress ($I_2$) it was higher than that under moderate ($I_3$) and severe ($I_4$) stresses (Figure 3). Germination rate of seeds for all ecotypes at the early phases of seed filling was low, but it was improved progressively with seed development up to 3-7 days after mass maturity (Figure 4) depending on irrigation intervals. Seeds from all ecotypes under moderate and severe stresses ($I_3$, $I_4$) had lower maximum germination rate. Seeds of Varamin and Malayer were germinated earlier than Isfahan ecotype (Figure 4).

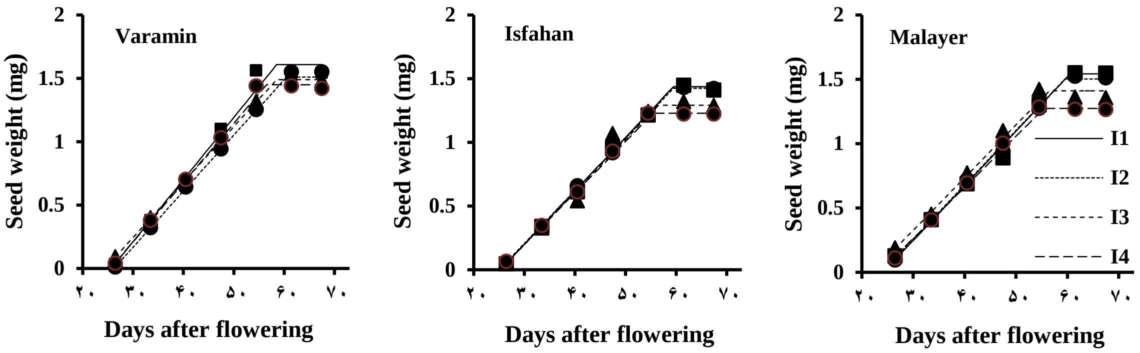

Figure 1. Changes in seed weight of three dill ecotypes at different stages of maturity under various irrigation levels; $I_1$, $I_2$, $I_3$, $I_4$: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

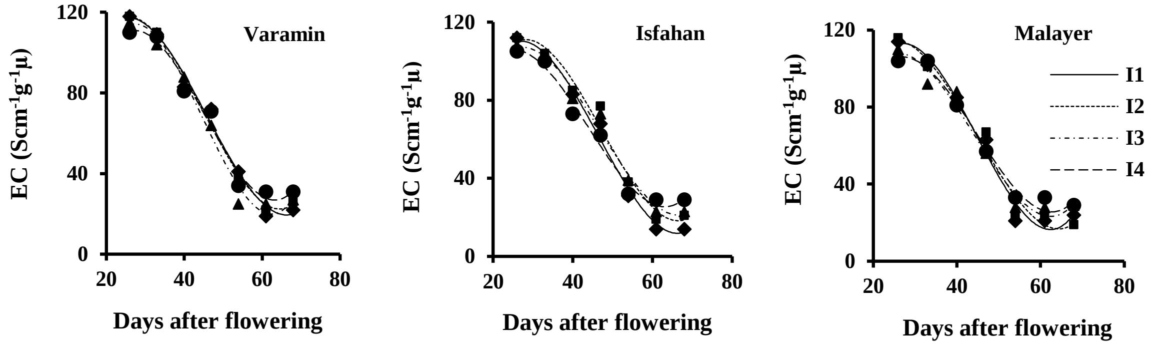

Figure 2. Changes in electrical conductivity (EC) of dill ecotypes at different stages of maturity under various irrigation levels; $I_1$, $I_2$, $I_3$, $I_4$: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

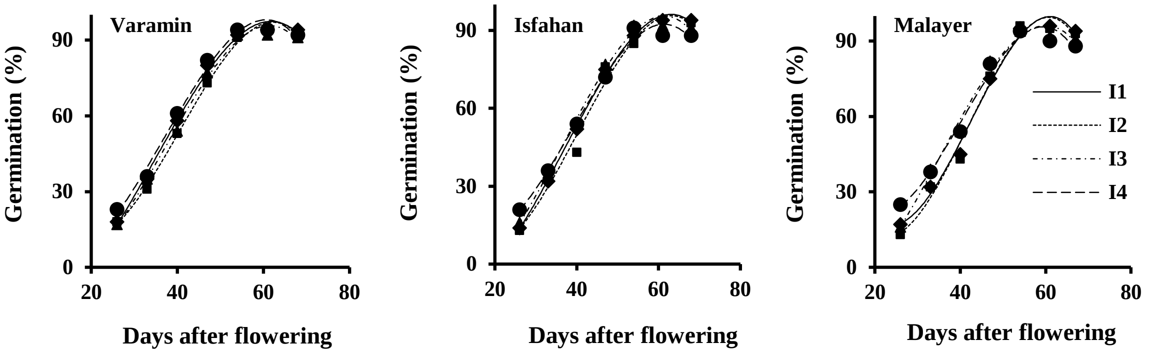

Figure 3. Changes in seed germination of dill ecotypes at different stages of maturity under various irrigation levels; $I_1$, $I_2$, $I_3$, $I_4$: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

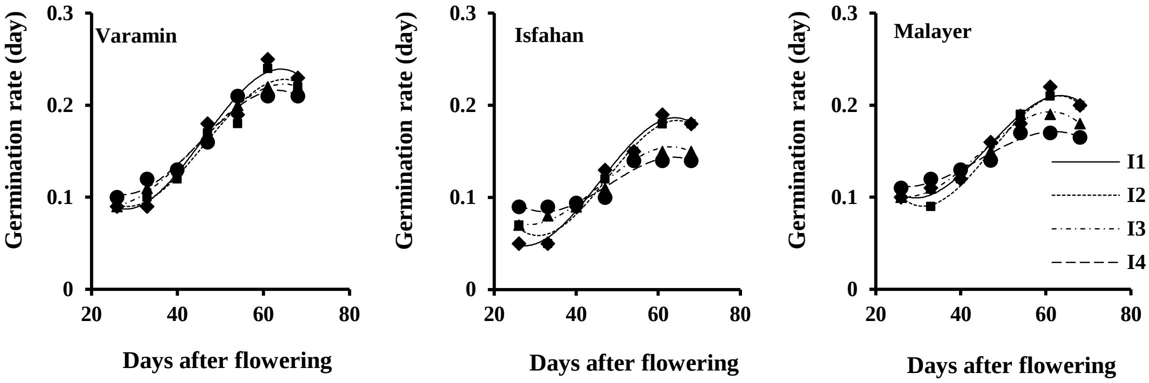

Figure 4. Changes in germination rate of dill ecotypes at different stages of maturity under various irrigation levels; $I_1$, $I_2$, $I_3$, $I_4$: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

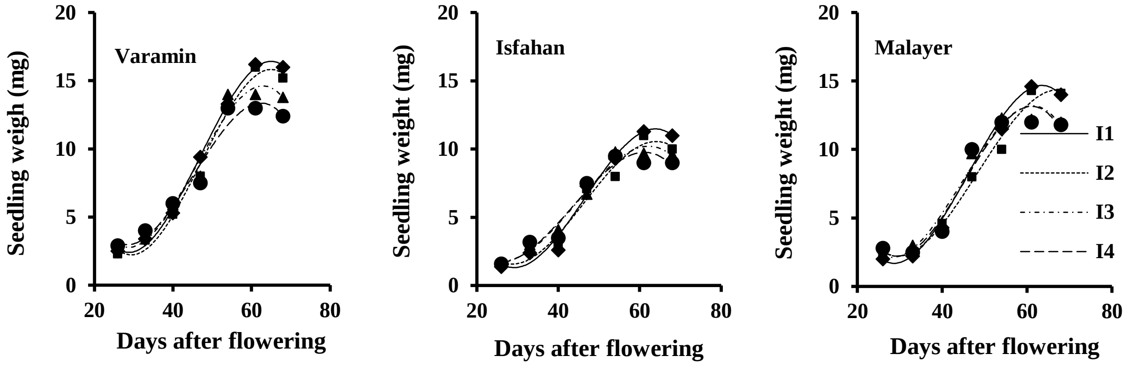

Figure 5. Changes in seedling dry weight of dill ecotypes at different stages of maturity under various irrigation levels; $I_1$, $I_2$, $I_3$, $I_4$: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Similar to changes in germination rate, dry weight of seedlings for all ecotypes at the early phases of seed filling was low, but it was augmented with developing seed maturity.

Maximum seedling dry weight under was obtained at 61-67 days after flowering or 3-8 days after mass maturity, depending on water supplies and ecotypes. However, seedling dry weight of seeds from later harvests gradually decreased. The largest seedlings were obtained from Varamin seeds, followed by Malayer seeds (Figure 5).

## Discussion

The principal reasons for reduction of seed weight under water stress (Figure 1) are declining net photosynthesis due to metabolic disruptions, oxidative damages to chloroplasts and closure of stomata (Farooq *et al.* 2014). Reduction of seed weight under limited irrigations was also reported for sorghum (Jaberledar *et al.* 2017). Variation in seed mass of ecotypes under different levels of watering (Figure 1) may be resulted from genetic differences among them. High food storage levels in seeds were positively associated with seed quality (Sinder *et al.* 2016).

High electrical conductivity values of seed lots at the early and later harvests (Figure 2) were probably due to immaturity and beginning of seed ageing on mother plant, respectively (Szemruch *et al.* 2015). Under severe water stress ($I_4$), as a result of lipid peroxidation in cell membranes (DaCosta and Huang 2007), protein degradations (Flick and Kaiser 2012), as well as loss of ribosomes (Woodstock *et al.* 1985) and modification of respiration capability (Martim *et al*. 2009), electrolyte leakage of seeds for all ecotypes was higher than the other irrigation treatments. Similar results were observed for four safflower ecotypes under different irrigation intervals (Ghassemi-Golezani *et al.* 2016). Therefore, the sequential events of seed development on water stressed plants may vary from those developing on non-stressed plants (Egli 1998). Severe oxidative attacks can damage membrane organization with consequent loss of cellular compartmentation. The membrane integrity may be maintained by decreasing free radical production and increasing the activities of anti-oxidants (Leprince *et al.* 1993).

Enhancing cell division and enlargement and also increasing translocation of assimilates from the mother plant to the seeds during development (Wang and Ruan 2013) was led to a higher percentage and rate of seed germination (Figures 3 and 4). Rapid germination of the seeds (Figure 4) resulted in production of larger seedlings (Figure 5). The importance of mean germination time as an indicator of seedling emergence rate has been proved in many crop species (Matthews *et al.* 2012). The late germinating seeds will likely produce smaller seedlings at a given time from sowing, compared with seedlings from the fast germinating seeds (Finch-Savage and Bassel 2016). The decline of percentage (Figure 3) and rate of germination (Figure 4) and seedling dry weight (Figure 5) at later harvests could be associated with the beginning of seed aging on mother plant (Ellis and Pieta Filho 1992; Szemruch *et al.* 2015). Although responses of dill ecotypes to water limitation were different, normal irrigation resulted in increasing germination percentage (Figure 3) and rate (Figure 4) and also seedling dry weight (Figure 5) in all ecotypes. These results clearly suggest that the most vigorous seeds of this crop can be produced under well-watering. Similar results were reported for common onion (El Balla *et al*. 2013), sesame (Silva *et al.* 2016), wheat (Shahi *et al.* 2015) and safflower (Ghassemi-Golezani *et al.* 2016). Water shortage cause changes in cell membrane structure that affect seed quality and germination (Guo *et al.* 2017).

Maximum seed quality as determined by electrolyte leakage of seeds (Figure 2), germination percentage (Figure 3), germination rate (Figure 4) and seedling dry weight (Figure 5) was obtained 2-12 days after mass maturity with 15-20% moisture content, depending on irrigation intervals and ecotypes. This is strongly supported by reports on barley (Ellis and Roberts 1992), chickpea (Ghassemi-Golezani and Ghassemi 2013), cowpea (Eskandari 2012) and soybean (Ghassemi-Golezani *et al.* 2015).

## Conclusions

Seed quality of dill ecotypes enhanced with increasing seed filling on mother plant under various irrigation intervals. The results indicated that high quality seeds of dill can be produced under normal and limited levels of water availability, if the seeds are harvested 2-12 days after mass maturity and seed moisture content is about 15-20%. Nevertheless, water limitation can somewhat reduce seed vigor of dill ecotypes. The highest seed vigor was recorded for Varamin, followed by Malayer ecotypes. In contrast, the poorest vigor seeds were produced by Isfahan ecotype. These differences in seed vigor could be related with the genetic differences among the ecotypes.

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# تغییرات کیفیت بذر ارقام شوید (*Anethum graveolens*) در واکنش به محدودیت آب و زمان برداشت

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## چکیده

یک آزمایش اسپلیت پلات بر پایه بلوک‌های کامل تصادفی با سه تکرار در سال ۱۳۹۴ اجرا گردید تا بهترین مرحله برداشت سه اکوتیپ شوید (*Anethum graveolens*) شامل ورامین، اصفهان و ملایر تحت شرایط آبیاری متفاوت (آبیاری پس از ۷۰، ۱۰۰، ۱۳۰ و ۱۶۰ میلی متر تبخیر از تشتک تبخیر کلاس A) برای تولید بذرهای با کیفیت بالا تعیین شود. نتایج به روشنی نشان داد که کیفیت بذر همه اکوتیپ‌ها در فواصل مختلف آبیاری با پیشرفت نمو بذر روی گیاهان مادر افزایش یافت. حداکثر کیفیت بذر بر اساس هدایت الکتریکی مواد نشتی از بذر، درصد و سرعت جوانه زنی و وزن خشک گیاهچه بسته به اکوتیپ و فواصل آبیاری در حدود ۱۲-۲ روز بعد از رسیدگی وزنی حاصل شد. برداشت‌های زودهنگام به سبب نارسی و برداشت‌های دیرهنگام به دلیل پیری موجب کاهش جوانه‌زنی و قدرت بذر شدند. با کاهش فراهمی آب برای گیاهان مادر، قدرت بذر همه اکوتیپ‌ها به تدریج تنزل یافت. بنابراین، نتیجه‌گیری شد که آبیاری گیاهان شوید به ویژه در دوره پر شدن بذرها می‌تواند سبب بهبود کیفیت آن‌ها شود. با این حال، بهترین محتوای رطوبت بذر برای برداشت گیاهان شوید و تولید بذرهای با کیفیت بالا در حدود ۲۰-۱۵٪ است. قوی‌ترین بذرها بر اساس سرعت جوانه‌زنی و وزن خشک گیاهچه توسط اکوتیپ‌های ورامین و سپس ملایر تولید شد.

**واژه‌های کلیدی:** جوانه‌زنی؛ رسیدگی؛ شوید؛ فاصله آبیاری؛ کیفیت بذر