

## Physiological Quality of Soybean Seeds Affected by Water and Light Deficits

Kazem Ghassemi-Golezani<sup>1\*</sup>, Javad Bakhshi<sup>1</sup>, Bahareh Dalil<sup>2</sup> and Mohammad Moghaddam Vahed<sup>3</sup>

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<sup>1</sup>Department of Plant Eco-physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

<sup>2</sup>Department of Agriculture, Payame Noor University, 19395-3697, Tehran, Iran

<sup>3</sup>Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

\*Corresponding author; Email: [golezani@gmail.com](mailto:golezani@gmail.com)

### Abstract

In order to determine the best developmental stage of soybean at which maximum seed quality is attained under different shadings ( $S_1$ ,  $S_2$ ,  $S_3$ : 0, 35 and 75% shade, respectively) and irrigation treatments ( $I_1$ ,  $I_2$ ,  $I_3$ ,  $I_4$ : irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively), two split plot experiments using randomized complete block design with three replications were conducted in 2011 and 2012. Seeds were harvested at five day intervals in eight stages. Maximum seed weight (mass maturity) was achieved at 41-54 days after flowering. Seed filling duration decreased with decreasing water supply, but it was increased by shading. As a result, maximum seed weight, germination rate and seedling dry weight of seeds from shaded plants were higher than those of unshaded plants. However, minimum electrical conductivity and maximum germination percentage of seeds from shaded and unshaded plants under different irrigation treatments were almost similar. Maximums of seed quality parameters were obtained 1 to 9 days after mass maturity, depending on the light availability and irrigation intervals. At earlier harvests, because of immaturity, and at later harvests, due to aging, seed physiological quality was low. It was concluded that high quality seeds of soybean can be produced under different irrigation and shading treatments, providing that the seeds are harvested at about 16-20% moisture content.

**Keywords:** Irrigation; Seed filling; Seed quality; Shading; Soybean

### Introduction

Successful crop production in any environment depends initially on the quality of the seeds being sown. The term 'seed physiological quality' is used in practice to describe the value of a seed lot for its intended purpose and includes germination and vigor (Ghassemi-Golezani *et al.* 2011a). Seed quality may influence grain yield of crop through both indirect and direct effects. The indirect effects include those on percentage emergence which influence yield by altering plant population density and spatial arrangement. Direct effects are those on emergence rate which influence seedling vigor and uniformity (Ghassemi-Golezani 1992).

Production of high quality seeds depends upon the appropriate time of harvest. In general, seed

crops should be harvested when seed quality is maximal (Vidigal *et al.* 2011). Harvesting too early may result in low yield and quality, because of the partial development of essential structures of seeds. Whereas, harvesting too late may increase the risk of shattering and decrease the quality of seeds due to aging (Ghassemi-Golezani and Mazloomi-Oskooyi 2008; Ghassemi-Golezani and Ghassemi 2013). Adverse environmental conditions such as raining may also result in sprouting of seeds on mother plants (Wang *et al.* 2008). Thus, precise information on optimum stage of harvest based on physiological indices could enable the seed producer to overcome adverse effects of environment and thereby avoiding severe yield losses (Tonapi *et al.* 2006).

According to some researchers (Harington 1972; Tekrony and Egli 1997; Ghassemi-Golezani *et al.* 2011b), mass maturity (end of seed filling phase) described as physiological maturity is a good sign of achieving maximum seed quality on the mother plant. Thereafter seeds begin to age, losing viability and vigor. In contrast, many reports on various crops showed that the quality of seeds on the mother plant continued to improve for some time beyond the end of seed filling phase (Ellis and Pieta Filho 1992; Demir and Ellis 1992; Ghassemi-Golezani and Mazloomi-Oskooyi 2008; Ghassemi-Golezani and Hosseinzadeh-Mahootchy 2009; Ghassemi-Golezani *et al.* 2011a; Ghassemi-Golezani and Ghassemi 2013).

Environmental stresses such as water deficit, low or high temperatures, nutrient deprivation and shading can occur during seed development. Sometimes more than one stress may be experienced by plants and the effect of these on seed development may be magnified and be greater than the sum of the individual stresses. Reported effects of environmental stresses during seed development on seed quality are inconsistent (Bewley and Black 1994). It was reported that radiation deficiency has little or no significant effect on germination ability or longevity in barley (Pieta Filho and Ellis 1991). Ghassemi-Golezani and Ghassemi (2013) also showed that seed quality as determined by germination percentage, germination rate and seedling dry weight were not significantly affected by water stress in chickpea. In contrast, Ghassemi-Golezani *et al.* (2012) found that water deficit during grain filling led to significant reduction in seed quality of soybean. Since, we did not find any report on the effect of

water and shade stresses on seed quality of soybean, this research was aimed to determine the interactive effects of water limitation and shading on seed quality development of soybean.

### Materials and Methods

Seeds of soybean were obtained from Moghan Agro-Industrial Company, Moghan, Iran. The seeds were treated with 2 g/kg Benomyl before sowing. Two field experiments were carried out at the Research Field of the University of Tabriz, Iran (latitude 38.050 N, longitude 46.170 E, altitude 1360 m above sea level) in 2011 and 2012. The climate is characterized by mean annual precipitation of 245.75 mm, mean annual temperature of 10 °C, mean annual maximum temperature of 16.6 °C and mean annual minimum temperature of 4.2 °C. The experiments were arranged as split plot based on randomized complete block design with three replications. Factors were four irrigation treatments (I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, I<sub>4</sub>: irrigation after 60, 90, 120 and 150 mm evaporation from class A pan, respectively) in main plots and three shading levels (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>: 0%, 35% and 75% shade, respectively) in sub-plots. Shading nets were spread over an iron framework (3 m x 3 m), 1.5 m above the soil to ensure good ventilation and full cover of the corresponding plots immediately after seedling establishment up to maturity. Seeds were hand sown on 6 May 2011 and 14 May 2012 in 4 cm depth of soil.

Each plot consisted of 10 rows of 3 m length, spaced 25 cm apart. Seeding rate was 64 seeds/m<sup>2</sup>. 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 seed formation, 10 plants from each plot were harvested at 5 day intervals in eight stages. Then seeds were detached from pods and seed moisture content was determined in accordance with ISTA (2010) rules, using two replicates of 5 g seeds. Subsequently, seeds were air dried at 18-20 °C and mean seed weight of each sample was determined. A two piece linear regression model was used to evaluate seed dry weight accumulation:

$$W = \begin{cases} a + bt & t < tm \\ b + btm & t \geq tm \end{cases}$$

where  $W$  is seed weight,  $a$  is the intercept,  $b$  is the slope,  $t$  is days after flowering and  $tm$  is the end of seed filling period (time of mass maturity). The remaining seed samples within separate sealed bags were kept in a refrigerator at  $4 \pm 1$  °C.

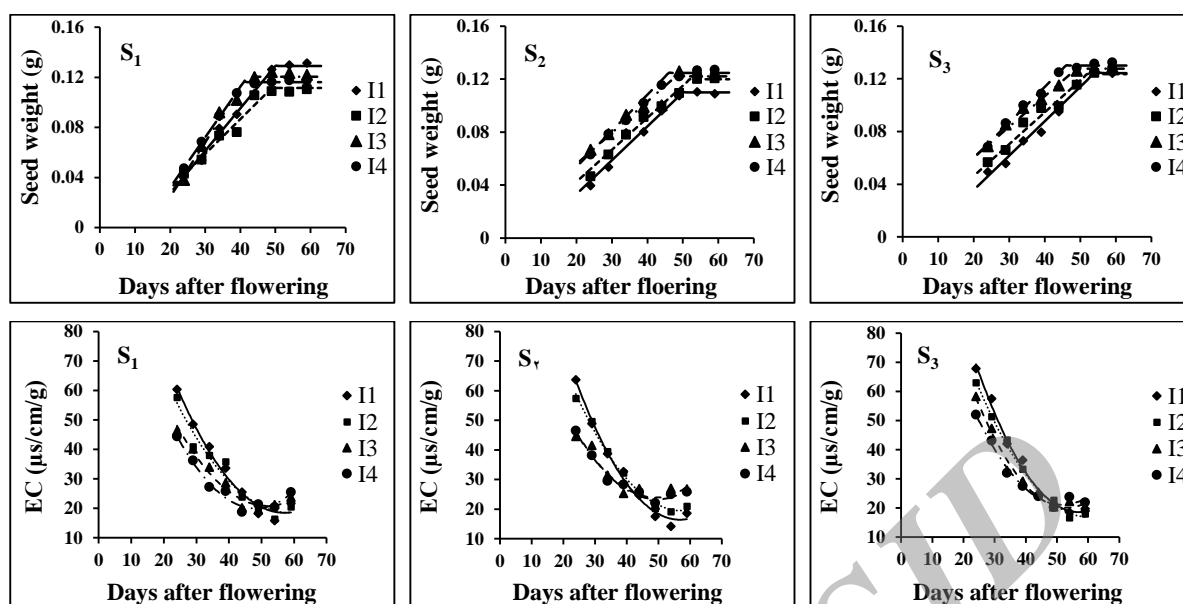
Two replicates of 50 seeds from each sample were weighed ( $SW_1$ ,  $SW_2$ ) and then seeds of each replicate immersed in 250 ml deionized water in a container at 20 °C for 24 hours. The seed-steep water was then gently decanted and EC was measured, using an EC meter ( $EC_1$ ,  $EC_2$ ) and mean conductivity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

Seed quality tests were carried out at the Seed Technology Laboratory of the University of Tabriz. Three replicates of 25 seeds from each sample were tested for germination between double layered rolled filter papers. The rolled papers with seeds were put into the plastic bags to avoid moisture loss. These rolled papers were

incubated at  $20 \pm 1$  °C for 8 days. Germinated seeds were recorded every 24 hours for 8 days. Rate of seed germination was calculated according to Ellis and Roberts (1980). At the end of this test (8 days), number of normal and abnormal seedlings was counted (ISTA 2010) and percentages of germination were calculated. Normal seedlings were then dried in an oven at 80 °C for 24 hours (Perry 1977) and mean seedling dry weight (SDW) for each replicate was determined. Excel software was used to fit regression lines on the mean data (means of two years and three replicates).

## Results

Regression lines clearly indicated that mean seed weight of soybean under different irrigation and shading treatments increased with increasing grain filling period up to a point where maximum seed weight was achieved. Thereafter, no changes in seed weight were observed. Mass maturity (end of seed filling) under  $I_1$ ,  $I_2$ ,  $I_3$  and  $I_4$  were achieved at 50, 49, 42 and 41 days after flowering for plants under  $S_1$  (full sunlight), at 50, 52, 48 and 47 days after flowering for plants under  $S_2$  (35% light interception) and at 54, 52, 49 and 46 days after flowering for plants under  $S_3$  (25% light interception), respectively. In general, seed filling duration was decreased by water stress, but it was increased by shading. As a result, seeds of shaded plants under water stress were larger than those of unshaded plants. However shading reduced maximum seed weight under well watering (Figure 1).



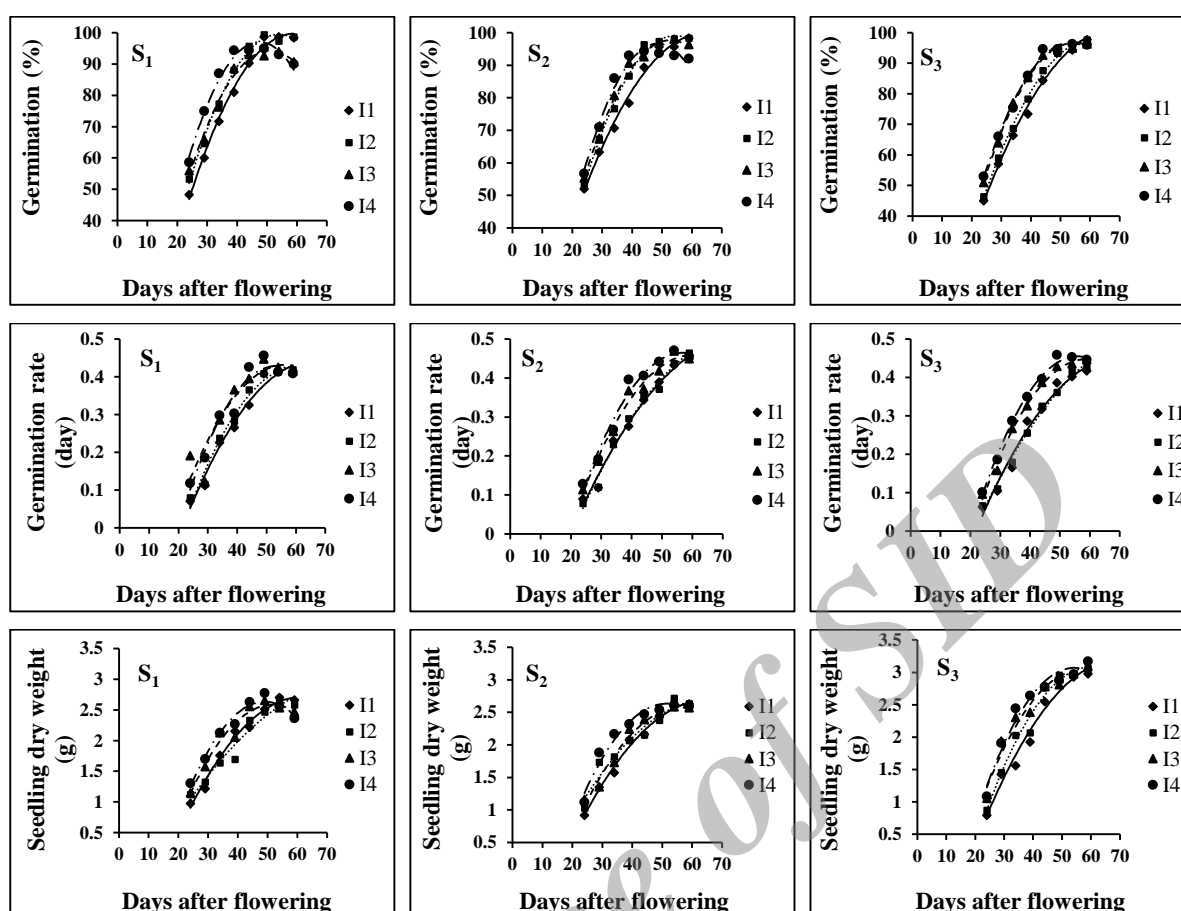
**Figure 1.** Changes in mean seed weight and electrical conductivity (EC) of seed leachates of soybean under different irrigations (I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, I<sub>4</sub>: Irrigation after 60, 90, 120 and 150 mm evaporation, respectively) and shadings (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>: 0%, 35% and 75% shading, respectively).

The data are means of two years and three replicates.

Electrical conductivity (EC) of seed leachates at early stages of seed development under different irrigation and shading treatments was very high, but sharply decreased with improving seed development. Electrolyte leakage for seeds of water stressed plants was lower than that for well irrigated plants at the early harvests. Consequently, minimum electrical conductivity of these seeds achieved earlier than the others, but in most cases there was no considerable difference in minimum values among seeds from shaded and unshaded plants under different irrigation treatments. Minimum electrical conductivity of the seeds was occurred at about 1-8 days after mass maturity, depending on irrigation and shading treatments. Seed moisture content at this stage was 16-20%. Thereafter, electrical conductivity (EC) of seed

leachates gradually increased up to final harvest (Figure 1).

Percentage of seed germination at early stages of seed growth was very low, but it was increased with enhancing seed reserves. Maximum seed germination percentage under I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub> and I<sub>4</sub> was obtained at 6, 3, 7 and 5 days after mass maturity for plants under S<sub>1</sub>, at 7, 2, 4 and 2 days after mass maturity for plants under S<sub>2</sub> and at 5, 7, 3 and 6 days after mass maturity for plants under S<sub>3</sub>, respectively. Thereafter, germination percentage of seeds from plants of I<sub>3</sub> and I<sub>4</sub> treatments decreased under S<sub>1</sub> and S<sub>2</sub>, but no considerable changes were occurred for those under S<sub>3</sub>. Maximum germination percentages of seeds from shaded and unshaded plants under different irrigation treatments were almost similar (Figure 2).



**Figure 2.** Changes in germination percentage and rate and seedling dry weight of soybean seeds produced under different irrigations (I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, I<sub>4</sub>: Irrigation after 60, 90, 120 and 150 mm evaporation, respectively) and shadings (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>: 0%, 35% and 75% shading, respectively).

The data are means of two years and three replicates.

Germination rate of soybean seeds under different irrigation and shading treatments increased with increasing grain filling period, and maximum value of this quality parameter was obtained at 5 to 9 days after the end of seed filling. Thereafter, in most cases, no considerable changes in seed germination rate were observed. Maximum germination rate of seeds from shaded plants was higher than that of unshaded plants under well and limited irrigation conditions (Figure 2).

Seedling dry weight of soybean for different irrigation and shading treatments increased with progressing seed development up to 3 to 9 days

after the end of seed filling. Seedling dry weight of seeds produced from S<sub>1</sub> and S<sub>2</sub> plants under limited irrigations, particularly under severe water deficit (I<sub>4</sub>) decreased at later stages of seed development. Maximum seedling dry weight of seeds from the most shaded plants (S<sub>3</sub>) was considerably higher than that of seeds produced under full sunlight (S<sub>1</sub>) and low shading (S<sub>2</sub>) under all irrigation treatments (Figure 2).

### Discussion

Maximum seed dry weight (mass maturity) under water stress and full sunlight attained earlier than

that under well-watering and shade (Figure 1). It is suggested that drought stress accelerates leaf senescence and reduces the duration of seed filling and weight, presumably by reducing photosynthesis and assimilate supply to developing seeds (Fageria 2013). Stimulation of seed maturity under water limitation was also reported for barley and wheat (Mc Master and Wilhelm 2003), maize and sorghum (Farre and Faci 2006) and common bean (Ghassemi-Golezani and Mazloomi-Oskooyi 2008). Nasrollahzadeh *et al.* (2011) also reported that seed filling duration of faba bean plants under shade was 3-4 days longer than that under full sunlight.

Increasing seed filling duration by shading, particularly under water stress could be the result of reducing temperature and water loss under this condition. This led to the production of larger seeds under water stress, but shading reduced maximum seed weight under well watering condition (Figure 1). Large seeds have greater supply of stored energy to support early seedling growth (Singh *et al.* 1972). It seems that larger seeds tend to produce more vigorous seedlings mainly due to larger embryos and longer nutritional support of the early seedling growth (Ghassemi-Golezani 1992). Therefore, seed size is considered to be a significant factor only during the early stages of growth.

Maximum seed quality as measured by electrical conductivity (Figure 1) of seed leachates, germination percentage, germination rate and seedling dry weight was obtained 1-9 days after mass maturity, depending on the factors and tests used in the experiment (Figure 2). Seed moisture content at these stages was 16–20%, varying

among irrigations and shades. These results contradict the hypothesis that seeds attain maximum quality at the end of seed filling phase (Harrington 1972), but compatible with the results reported for barley (Ellis and Pieta Filho 1992), common bean (Ghassemi-Golezani and Mazloomi-Oskooyi 2008), tomato (Demir and Samit 2001), faba bean (Ghassemi-Golezani and Hosseinzadeh-Mahootchi 2009), chickpea (Ghassemi-Golezani and Ghassemi 2013) and cowpea (Eskandari 2012).

Maximum seed physiological quality as measured by different tests was almost similar for seeds produced under different irrigation treatments (Figures 1 and 2). Therefore, high quality seeds of soybean can be produced under different watering conditions, provided the seeds are harvested at maximum quality with about 16-20% moisture content. Similar results were reported for faba bean (Ghassemi-Golezani and Hosseinzadeh-Mahootchi 2009). Low quality of seeds at early harvests was due to immaturity. Seed development is characterized by numerous cell divisions and differentiation of organs, acquisition of assimilates from the mother plant and consequently a substantial increase in seed weight. Reduction of seed quality at delayed harvests, particularly when seed quality was evaluated by electro-conductivity test (Figure 1) is attributed to the beginning of seed aging on parent plant (Ellis and Pieta Filho 1992).

Although minimum electrical conductivity (Figure 1) and maximum germination percentage for seeds of unshaded and shaded plants were similar, maximum seed germination rate and seedling dry weight were enhanced by shading (Figure 2). This means that radiation deficiency

can improve seed and seedling vigor through enhancing seed weight and germination rate, particularly under water stress (Figures 1 and 2). Thus, the stage of achieving maximum seed quality may be influenced by environmental conditions, harvest time and quality test. Nevertheless, high quality seeds of soybean could be produced, if seeds were harvested shortly after mass maturity.

Seed quality at early and late harvests is low, because of immaturity and aging, respectively. High quality seeds can perform well in the field, ensuring optimum stand establishment and satisfactory yield under a wide range of environmental conditions (Ghassemi- Golezani *et al.* 2010).

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