Effects of Different Priming Treatments on Seed Germination Properties, Yield Components and Grain Yield of Lentil (*Lens culinaris* Medik.)

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Abstract

An experiment was conducted under laboratory and field conditions in order to evaluate the effects of different priming treatments, specifically KNO₃ (1%), KCl (2%), KH₂PO₄ (1%), ZnSO₄ (0.05%), PEG-6000 (2%), IBA (100 ppm), Mannitol (4%), GA₃ (100 ppm) and distilled water, on seed germination properties and several agro-morphological plant characteristics of red lentil. Seeds not primed were used as a control. GA₃ treatment increased shoot length. The control (non-primed seeds) treatment resulted in increased seedling root number and length. Distilled water, ZnSO₄, and control treatments increased germination rate and percentage. In the pot experiments, GA₃ treatment increased plant height and seedling emergence rate, whereas KCl treatment improved the number of nodules, as well as root and shoot dry weight when compared to the control. ZnSO₄ treatment increased yield components and grain yield in field conditions. The results of this study showed that ZnSO₄, GA₃ and PEG-6000 seed priming treatments may be useful tools due to their positive effects on germination rate, germination percentage, yield component and grain yield of lentil.

Keywords: germination, grain yield, lentil, seed priming

Introduction

Lentil (*Lens culinaris* Medik.) is a diploid (2n=2x=14), self-pollinated (autogam) and annual species of grain legume. Lentil is one of the most ancient crops, first cultivated 7,000-8,000 years ago (Cubero, 1981; Lev-Yadun *et al.*, 2000). Lentil is an important source of protein, minerals and vitamins in the human diet (Karakoy *et al.*, 2012; Muehlbauer *et al.*, 2006; Solanki *et al.*, 1999). Lentil is cultivated as a rainfed crop on 4.3 million hectares, which yields 4.9 million tons; in Turkey, it is grown on 281 thousand hectares and yields 47 thousand tons (FAO, 2013). Biotic and abiotic stress conditions, such as drought, cold, insufficient rainfall, salt stress and disease, are the most common restrictive conditions in lentil-growing areas and negatively affect lentil production. Germination and seedling emergence stages are critical for crop production; rapid and uniform field emergence is essential to achieve high yield and uniform plant stands, resulted in early maturity and reduced disease attack (Ali *et al.*, 2005; Cheng and Bradford, 1999; Subedi and Ma, 2005). Finding ways to overcome environmental stresses, such as inadequate moisture during seed germination, is important for an economic crop production (Ashraf and Rauf, 2001).

Much research has been conducted involving germination and growth within uniform seed plantings. Pre-sowing seed treatments (seed priming) before germination and metabolic activity in grass seed have been reported to have a positive effect on seedling growth (Bradford, 1986). Seed priming treatments resulted in positive effects on many field crops, such as wheat, sugar beet, maize, soybean and sunflower (Parera and Cantliffe, 1994; Saglam *et al.*, 2010). Seed priming treatment activates some metabolic activity without actual germination. For priming treatment, seeds are immersed in a solution with high osmotic potential. In this case, sufficient water uptake for seed germination was prevented, thus suspending the seeds in the lag phase (Ashraf and Foolad, 2005; Taylor *et al.*, 1998). Seed priming treatments are widely used to reduce the time between the planting date and the seedling formation period, and to provide uniform plant growth (Parera and Cantliffe, 1994). Water deficit pose an increasing problem worldwide, especially in arid and semi-arid zones where the water intake period for seed germination is limited. Therefore, seedling stand establishment is dependent upon rapid and uniform germination (Fischer and Turner, 1978; Yordanov *et al.*, 2000).

The most common seed priming treatments used to increase seed germination and synchronization are osmopriming (immersing the seeds in solutions with osmotic potential), halopriming (placing the seeds in a salt solution), hydropriming (placing the seeds in water), matripriming (placing the seeds between saturated jute mat layers) and hardening (alternately soaking and drying the seeds) (Basra *et al.*, 2003; Khan, 1992; McDonald, 2000). Ghassemi-Golezani *et al.* (2008) reported that hydropriming treatments increased the weight of the seedling root, the germination rate, as well as shoot, root and seedling dry weight. KNO₃ and PEG treatments increased germination percentages when compared to the control. Saglam...
et al. (2010) reported priming treatments decreased the effects of water stress, and Ghassemi-Golezani et al. (2013) observed that hydropriming treatments to lentil seeds increased the plant height, number of pods and number of seeds per plant, the 1,000 grain weight, the biological yield, the grain yield and the harvest index when compared to the control.

The aim of this study was to research the effects of osmo- and hydro-priming treatments to lentil seeds, to analyse the influence of sucha treatment over seed germination properties and some plant characteristics, yield components and grain yield in an experiment conducted with field conditions.

Materials and methods

Seed materials and priming treatments

This study was conducted at the Vocational School of Kozan, Çukurova University. Seeds of red lentil cultivar ‘Firat 87’ were used. Newly harvested seeds were kindly obtained from Dicle University, Agricultural Faculty, Field Crops Department. Solutions of KNO$_3$ (1%), KCl (2%), KH$_2$PO$_4$ (1%), ZnSO$_4$ (0.05%), PEG-6000 (20%), IBA (100 ppm), Mannitol (4%), GA$_3$ (100 ppm) and distilled water were used for priming treatments (Ghassemi-Golezani et al., 2008; Giri and Schillinger, 2003; Michel and Kaufmann, 1973; Yari et al., 2011). All priming media were prepared in distilled water. Unprimed seeds were used as a control. The seeds were divided into ten sub-samples of 95 g each, and one sub-sample was used as the control (unprimed). The nine other sub-samples were used for priming treatments. Lentil seeds were primed separately in 1,000 mL solutions of each respective priming agent at 20 °C for 12 h under dark conditions in an incubator (Ghassemi-Golezani et al., 2008). All primed seeds were then removed from the priming media and the surface sterilized (including control seeds) with 2% sodium hypochlorite (NaOH). After the surface sterilization, seeds were washed with distilled water and dried on paper towels at room temperature, under ventilated conditions, until they regained their original moisture content (Ghassemi-Golezani et al., 2008).

These primed seeds were used for germination tests in laboratory, pot and field experiments. Electrical conductivity, pH and seed weight gain (%) after priming treatments were analyzed with specific methodology.

Germination tests

Germination tests were carried out according to standard protocols established by the International Seed Testing Association (ISTA). The experimental units were arranged in a completely randomized design with four replications. Twenty-five seeds from each of the priming treatments were placed on the moistened filter papers in petri dishes and germinated in an incubator at 10 ± 1 °C. Germination of seeds (protrusion of radicle by 2 mm) was recorded at daily intervals over the course of the 21 days (Ghassemi-Golezani et al., 2008). The seed germination rate was computed using the formula:

$$\frac{\sum n}{\sum D_n}$$

where $n$ is the number of seeds germinated on day $D$, and $D$ is the number of days counted from the beginning of the test.

For further analyses, 21-day-old seedlings were cut from the cotyledon level, and the leaved stem and root were dried at 75 ± 1 °C for 24 hours in the etuve (Ghassemi-Golezani et al., 2008).

Pot experiments

Pot experiments were arranged according to a completely randomized design with four replications. Ten seeds from each of the priming treatments were sown at a depth of 3-4 cm in plastic pots. Each pot had a volume of 1,500 mL and contained 1,200 mL of soil mixture comprised of 1/3 sand, 1/3 peat and 1/3 field soil. Water retention capacity was determined before sowing for the 1,200 mL soil mixture. Pots were irrigated once weekly until the soil reached 50% of its water-holding capacity. Germination rate (1/day) and percentage (%) were computed daily by counting all individual seedlings during the 21 days after germination started. The onset of the flowering stage, plant height (cm), number of nitrogen fixed root-nodules, shoot dry weight and root dry weight (g) were determined on five of ten plants in each pot.

Field experiments

Field experiments were arranged according to a randomized complete block design with three replications. Each plot consisted of 4 rows, each of 3 m long with 20 cm row spacing and 180 seeds planted per row. The seeds were planted in wet soil, and germinated seedlings were counted in each 2 m middle section of the two middle rows of each plot to determine seedling numbers per square meter.

Using ten randomly selected plants, plant height (cm), biological yield (g/plant), number of branches per plant, number of pods per plant, weight of pods per plant (g), number of seeds per plant, grain yield per plant (g/plant) and 1,000 grain weight (g) were measured. The middle two rows of each plot were hand-harvested to detect grain yield (kg/ha).

Results

Electrical conductivity, pH and seed weight gain (%) after priming treatments are listed in Table 1.

The results of the analysis of variance (ANOVA) for some plant characteristics in laboratory, pot and field conditions for seed priming effects on lentil seeds are included in Table 2. There were significant differences among the different priming treatments for all germination traits under laboratory conditions. The effects of seed priming on plant traits observed in pot conditions were significant, with the exception of root length (Table 2). There were significant differences in the agromorphological plant characteristics among seed priming treatments, with the exception of plant height, in the field experiments.

Laboratory experiment

There were significant differences among seed priming treatments with respect to shoot length, length of seedling root, number of seedling roots, shoot dry weight, root dry weight and germination rate and percentage (Table 3). The lowest shoot and seedling root lengths were observed in seeds treatment with IBA (3.35 and 2.23 cm, respectively), while the greatest shoot length was observed in seeds that received the GA$_3$ treatment (6.71 cm). GA$_3$ treatment increased the shoot length with 34.2% when compared to the control.

The control, distilled water and PEG-6000 increased seedling root length, while IBA treatment decreased root length (Table 3). The lowest seedling root number was obtained by treating seeds with GA$_3$ and KH$_2$PO$_4$ (1.39 and 1.60, respectively). The control, distilled water and PEG-6000
Table 1. Electrical conductivity and pH value of priming media and weight increase of primed seeds

| Priming media       | Before seed immersion | After seed immersion | Weight increase of primed seeds (%) |
|---------------------|-----------------------|----------------------|-------------------------------------|
|                     | Electrical conductivity (µS/cm/g) | pH | Electrical conductivity (µS/cm/g) | pH |                        |
| KNO₃ (1%)           | 14.05                 | 8.0 | 14.01                  | 5.6 | 78.2                   |
| KCl (2%)            | 39.40                 | 6.6 | 39.10                  | 6.0 | 72.6                   |
| KH₂PO₄ (1%)         | 7.41                  | 4.8 | 7.40                   | 5.4 | 72.3                   |
| ZnSO₄ (0.05%)       | 460.00                | 6.2 | 490.00                 | 5.5 | 78.2                   |
| PEG-6000 (20%)      | 86.00                 | 6.6 | 96.60                  | 6.6 | 47.7                   |
| IBA (100 ppm)       | 18.72                 | 4.8 | 38.50                  | 4.9 | 77.0                   |
| Mannitol (4%)       | 1.27                  | 8.0 | 29.25                  | 7.0 | 73.3                   |
| GA₃ (100 ppm)       | 59.30                 | 4.6 | 65.00                  | 4.8 | 79.1                   |
| Distilled water     | 1.50                  | 7.8 | 45.80                  | 6.6 | 79.8                   |

Table 2. Analysis of variance (ANOVA) for seed priming effects on lentil seed germination and plant characteristics in laboratory, pot and field conditions

| Plant characteristics | Mean square | Plant characteristics | Mean square | Plant characteristics | Mean square |
|-----------------------|-------------|-----------------------|-------------|-----------------------|-------------|
| Shoot length          | 4.24090**   | Plant height          | 33.95**     | Number of plants per square | 860.613**   |
| Seedling root length  | 8.03144**   | Root length           | 10.93       | Plant height          | 12.2877     |
| Number of seedling roots | 3.35770**    | Number of root nodules | 31.28**     | Biological yield      | 0.539404**  |
| Shoot dry weight      | 0.0000029** | Shoot dry weight      | 0.0060*     | Number of branches per plant | 35.8942**   |
| Root dry weight       | 0.0000034** | Root dry weight       | 0.0012**    | Number of pods per plant  | 78.1829**   |
| Rate of germination   | 0.005744**  | Germination rate      | 0.0052**    | Weight of pods per plant | 0.100112**  |
| Germination percentage| 51.1288**   | Germination percentage | 1389.17**   | Number of seedlings per plant | 102.832** |
|                       |             |                       |             | Grain yield per plant   | 516.902**   |
|                       |             |                       |             | Thousand kernel weight  | 102.832**   |
|                       |             |                       |             | Grain yield             | 516.902**   |

* Statistically significant at p ≤ 0.05; ** Statistically significant at p ≤ 0.01

Table 3. Seed priming effects on some germination and seedling properties in laboratory conditions

| Priming media | Shoot length (cm) | Root length (cm) | Number of seedling root | Shoot dry weight (mg) | Root dry weight (mg) | Germination rate (1/day) | Germination percentage (%) |
|---------------|-------------------|------------------|-------------------------|-----------------------|----------------------|--------------------------|-----------------------------|
| KNO₃          | 3.58 ef*          | 3.93 d           | 2.33 c                  | 2.90 d                | 2.60 c               | 0.161 bc                 | 92 a                        |
| KCl**         | --                | --               | --                      | --                   | --                   | 0.082 g                  | 26 c                        |
| KH₂PO₄        | 3.87 de           | 4.48 ed          | 1.60 d                  | 3.80 c                | 2.40 c               | 0.144 e                  | 81 b                        |
| ZnSO₄         | 4.70 bc           | 5.10 c           | 3.26 b                  | 4.30 abc              | 4.00 b               | 0.182 abc                | 97 a                        |
| PEG-6000      | 4.59 bc           | 6.15 b           | 3.26 b                  | 4.80 a                | 4.50 ab              | 0.168 cd                 | 100 a                       |
| IBA           | 3.35 f            | 2.23 e           | 3.93 a                  | 2.30 d                | 3.00 c               | 0.099 f                  | 95 a                        |
| Mannitol      | 3.64 ef           | 4.95 c           | 2.93 b                  | 4.00 bc               | 4.00 b               | 0.166 d                  | 97 a                        |
| GA₃           | 6.71 a            | 4.27 ab          | 1.39 d                  | 4.20 abc              | 2.80 c               | 0.180 bc                 | 94 a                        |
| Distilled water | 4.36 cd          | 6.30 ab          | 2.91 b                  | 4.60 ab               | 4.50 ab              | 0.196 a                  | 97 a                        |
| Control       | 5.00 b            | 6.86 a           | 3.96 a                  | 4.80 a                | 5.00 a               | 0.186 ab                 | 100 a                       |

* Different letters indicate a significant difference of p ≤ 0.05; ** Could not obtain data because of the seedlings killed at an advanced stage of germination

Table 4. Seed priming effects on several plant characteristics in pot experiment conditions

| Priming media | Plant height (cm) | Root length (cm) | Number of root nodules | Shoot dry weight (mg) | Root dry weight (mg) | Seedling emergence rate (1/day) | Seedling emergence percentage (%) |
|---------------|-------------------|------------------|------------------------|-----------------------|----------------------|---------------------------------|----------------------------------|
| KNO₃          | 14.15 e*          | 21.40            | 6.00 d                 | 198 b                 | 84 b                 | 0.162 bc                        | 87.5 a                          |
| KCl           | 17.41 bc          | 23.85            | 13.80 a                | 319 a                 | 121 a                | 0.108 c                         | 57.5 b                          |
| KH₂PO₄        | 14.48 de          | 24.10            | 8.85 cd                | 209 b                 | 79 bc                | 0.162 bc                        | 95.0 a                          |
| ZnSO₄         | 14.94 cde         | 21.10            | 7.38 cd                | 199 b                 | 64 bc                | 0.159 bc                        | 95.0 a                          |
| PEG-6000      | 14.68 cde         | 22.47            | 6.70 d                 | 186 b                 | 71 bc                | 0.166 b                         | 95.0 a                          |
| IBA           | 16.69 bcd         | 19.12            | 11.95 ab               | 205 b                 | 83 bc                | 0.123 bc                        | 97.5 a                          |
| Mannitol      | 17.02 bcd         | 21.02            | 12.10 ab               | 211 b                 | 77 bc                | 0.164 bc                        | 97.5 a                          |
| GA₃           | 23.93 a           | 20.92            | 7.70 cd                | 201 b                 | 66 bc                | 0.236 a                         | 45.0 b                          |
| Distilled water | 17.95 b          | 19.50            | 10.00 bc               | 189 b                 | 56 c                 | 0.133 bc                        | 92.5 a                          |
| Control       | 18.42 b           | 20.45            | 12.85 ab               | 234 b                 | 76 bc                | 0.120 bc                        | 95.0 a                          |

* Different letters indicate a significant difference of p ≤ 0.05; ** Could not obtain data because of the seedlings killed at an advanced stage of germination
Table 5. Seed priming effects on several plant characteristics in field experiment conditions

| Priming media | Number of plants per square meter | Plant height (cm) | Biological yield (g/plant) | Number of pods per plant | Number of seeds per plant | Grain yield per plant (g/plant) | 1000 grain weight (g) |
|---------------|----------------------------------|------------------|--------------------------|--------------------------|--------------------------|---------------------------------|---------------------|
| KNO3          | 217.5 d                          | 49.0             | 2.468 a                  | 11.13 ab                 | 6.60 cd                  | 0.277 ed                        | 35.77 b             |
| KCl           | 236.8 bed                        | 43.9             | 1.990 c                  | 15.30 a                  | 15.05 a                  | 0.488 ab                        | 26.09 cd            |
| KH2PO4        | 266.8 a                          | 43.8             | 2.046 bc                 | 3.80 d                   | 3.75 de                  | 0.363 bc                        | 23.47 d             |
| ZnSO4         | 244.3 abc d                      | 49.4             | 2.126 bc                 | 14.55 a                  | 18.25 a                  | 0.678 a                         | 37.17 b             |
| PEG-6000      | 229.5 cd                         | 45.3             | 1.452 d                  | 9.40 bc                  | 10.00 b                  | 0.376 bc                        | 37.60 b             |
| IBA           | 251.2 abc d                      | 45.4             | 1.452 d                  | 11.20 ab                 | 10.55 b                  | 0.388 bc                        | 35.80 b             |
| Mannitol      | 243.3 abc d                      | 45.8             | 2.031 bc                 | 8.56 bc                  | 4.00 de                  | 0.152 de                        | 37.45 b             |
| GA3           | 241.04 abc d                     | 43.8             | 2.226 b                  | 8.60 bc                  | 2.93 c                   | 0.058 e                         | 34.87 b             |
| Distilled water | 216.6 d                         | 46.9             | 1.333 d                  | 6.33 cd                  | 4.93 de                  | 0.133 de                        | 29.47 e             |
| Control       | 263.3 ab                         | 46.6             | 2.542 a                  | 10.10 bc                 | 8.30 bc                  | 0.353 bc                        | 42.57 a             |

* Different letters indicate a significant difference of p ≤ 0.05

Pot experiment

There were significant differences for all plant characteristics, with the exception of root length, among different priming treatments in the pot experiment (Table 4).

Of all the seed priming treatments used, the GA3 treatment significantly improved plant height, which increased 40% over the control and 30% over seeds primed with distilled water in pot conditions (Table 4). KCl treatment improved the number of nodules, as well as root and shoot dry weight. GA3 seed priming treatment increased the seedling emergence rate (0.236 1/day) when compared to the control and other priming treatments, while KCl treatment caused a decrease in the seedling emergence rate. GA3 and KCl priming treatments decreased the seedling emergence percentage when compared to the control (Table 4).

Field experiment

There were significant differences among priming media for all the plant characteristics observed in field conditions, with the exception of plant height (Table 5). The greatest number of plants per square meter was detected in plants grown from seeds treated with KH2PO4 (266.8), while the lowest number of plants was observed in plants grown from seeds that had undergone the distilled water treatment (216.6). The highest biological yields recorded were in the control group and the group that had received KNO3 treatment (2.562 and 2.468 g, respectively), while the lowest biological yield was observed in the group that received the distilled water treatment (1.333 g). The number of pods per plant ranged between 3.80-15.30. KCl and ZnSO4 treatments increased the number of pods per plant when compared to the control. Likewise, the number of seeds per plant increased when seeds were treated with ZnSO4 and KCl (18.25 and 15.05), respectively, while the lowest number of seeds per plant was observed in the group treated with GA3 (2.93 seeds/plant). Grain yield per plant ranged between 0.058 and 0.678 g (GA3 and ZnSO4, respectively). The lowest 1,000 grain weight was obtained by the control group (32.57 g), while the lowest was detected in those treated with KH2PO4 (Table 5).

KCl salts negatively affected the germination properties of lentil seeds in both laboratory and pot conditions. The reason for the decreased germination percentage may be explained by the toxic effect of KCl salts on germinating embryos, which caused the decreased germination percentage and deaths of the seedlings. Similar effects of KCl treatment on wheat seeds have been reported by Giri and Schillinger (2003) and Yari et al. (2011). In this research, GA3 treatment of lentil seeds caused an increase in plant height in both laboratory and pot conditions. Considering GA3, a plant growth hormone, its effect on promoting growth is slight. In addition, positive effects of GA3, KCl, and PEG-6000 treatments increased the seedling emergence rates (Tables 3 and 4). Positive effects of GA3 treatment were observed on the germination and seedling emergence rates (Tables 3 and 4). Positive effects of GA3 treatment were observed on the germination and seedling emergence rates (Tables 3 and 4). SD effect of GA3 treatment on wheat seeds have been reported by Giri and Schillinger (2003) and Yari et al. (2011). In this research, GA3 treatment of lentil seeds caused an increase in plant height in both laboratory and pot conditions. Considering GA3, a plant growth hormone, its effect on promoting growth is slight. In addition, positive effects of GA3, KCl, and PEG-6000 treatments increased the seedling emergence rates (Tables 3 and 4). Positive effects of GA3 treatment were observed on the germination and seedling emergence rates (Tables 3 and 4).
A greater number of nodules, along with increased root and shoot dry weight, following treatment of seeds with KCl may be explained by the low number of germinated seeds, but those that did germinate showed better development of root and shoot. The results of the laboratory and pot experiments showed that the IBA treatment decreased root length, while increasing the number and weight of roots. Decastro et al. (2000) reported that IAA, IBA and NAA seed treatments enhanced root formation and development. The positive effect of ZnSO\(_4\) seed treatment on the number of seeds and number of pods per plant and grain yield may be explained by the homogenizing effect of ZnSO\(_4\) on soil minerals at the root zone of plants and by its ability to facilitate nutrient uptake by plants. In addition, the higher anthracnose incidence that occurred during the growing season revealed that ZnSO\(_4\) treated plants exhibit greater resistance to the disease, which resulted in a slightly higher grain yield among seeds treated with ZnSO\(_4\) when compared to the control and seeds that underwent other priming treatments. However, more research is needed on this topic. These results are in agreement with results reported in Kaya et al. (2007), which demonstrated that Zn priming treatment on phaseolus seeds increased grain yield and yield components; Ajouri et al. (2004) also reported that Zn treatment to barley seeds increased germination and seedling development; and Marschner (1995) reported that higher Zn content of seeds at the germination stage increased resistance to soil-borne diseases.

Conclusions

As result of this study, it could be concluded that, with respect to germination properties, several plant characteristics, grain yield components and grain yield, GA\(_3\), PEG and ZnSO\(_4\) priming treatments should be considered, and further research is needed in order to obtain more conclusive results.

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