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Seed priming effects on germination and first growth of the medicinal plant *Achillea millefolium* L.

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ABSTRACT

This study evaluated the effects of seed priming on germination and growth of *A. millefolium* by means of laboratory and greenhouse experiments conducted during 2018 in Agricultural University of Athens. Treatments were GA₃ (400 and 800 ppm), potassium nitrate (2% and 4%), polyethylene-glycol (soaking for 12 and 24h) besides an untreated control. Experiment in Petri dishes revealed that GA₃ at 400 ppm, potassium nitrate (at concentration 2 and 4%) and PEG significantly increased germination percentage of *A. millefolium*, while germination rate was also significantly improved as a result of all seed priming techniques. In addition, due to the soil experiment, seedling emergence was significantly increased by GA₃ at 400 ppm, potassium nitrate (at both concentrations) and PEG compared with the untreated seeds. Dry biomass of the young seedlings was significantly enhanced by means of GA₃ (at 400 and 800 ppm), KNO₃ (4%) and PEG for 24 h, indicating the potential effect of seed priming on first growth as well. The results of the present study revealed the significant positive effects of seed priming on *A. millefolium* seed germination, seedling emergence and early growth.

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INTRODUCTION

New health challenges along with the observed reduction of efficacy and the increase of toxicity or side-effects of synthetic drugs increase the interest in herbal drugs and medicinal plants [1]. Medicinal plants are widely used for the production of drugs, food supplements, cosmetics and other products and therefore the interest is high, even there are significant differences on the rules and procedures between the countries of the European Union [2].

Achillea millefolium (yarrow) is a medicinal plant known since ancient years and a member of Asteraceae family. Its use was continuous and widespread, with reports that the seventh century AD the Slavic people used *A. millefolium* against several insects [3]. It is a plant used against dyspepsia, colic, diarrhea, hypertension, rheumatisms and for the treatment of many ailments due to the inflammatory antioxidant properties of the secondary metabolites [4,5]. Petrakou *et al.* (2020) found that *A. millefolium* was one of the medicinal plants with the highest relative importance and high use, in a large region of Greece and other Mediterranean countries [6].

Many medicinal plants have problems in seed germination and field performance and consequently, suggesting seed quality enhancement methods is necessary [7]. Seed priming is one of the methods of seed treatment for quality improvement and seed enhancement in different crops and can be defined as the process of controlled hydration of seeds [8,9]. In many cases, seed priming enhances rapid and uniform germination and seedling vigor in several crops and under a wide range of soil and climatic conditions [10,11]. There are several priming techniques used for seeds such as chemoprime, thermoprime, hydropriming and osmoprime [10,12]. Polyethylene glycol (PEG), gibberellins (GA) and potassium nitrate (KNO₃) are well documented chemicals which increase the seed germination of several plant species [13,14].

Low seed germination and a considerable variation between seed batches of *A. millefolium* have been reported [15,16]. Therefore, the objective of this research was to determine the effects of different seed priming treatments on seed germination parameters and seedling emergence and early growth of the medicinal plant, *A. millefolium*.

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MATERIALS AND METHODS

This study examined the effects of seed priming on germination and growth of *A. millefolium* by means of laboratory and greenhouse experiments conducted during 2018 (April to June) in Agricultural University of Athens. Seeds were collected by an experimental field in western Greece and properly stored until the establishment of the present experiments. Before the beginning of the experiment, the seeds were disinfected with 5% sodium hypochlorite solution for 2 min. After that, seeds were washed with distilled water and 25 of them were put in Petri dishes on sterilized Whatman papers. Seed priming treatments were gibberellic acid (GA_3) with dosages of 400 and 800 ppm, potassium nitrate (2% and 4%), polyethylene-glycol (PEG) 6000 for 12 and 24 h and control (untreated). The selection of the specific concentrations was based on previous preliminary tests. 5 ml of water was added to each dish and there were 4 dishes for each treatment and the untreated control. Germination experiments lasted 16 days and they were carried out in a completed randomized design under laboratory conditions, in growth chambers (Conviron T 38/Lb/AP) at constant temperature (25 °C) and total darkness. Seeds were considered germinated at the emergence of the radicle [17].

After the lapse of the experimental period (16 days), germination percentage and rate of germination were evaluated. Germination rate (GR) was determined using Maguire's index [18] as follows:

$$GR = (\text{Number of germinated seeds at first count / day of first count}) + (\text{Number of germinated seeds at second count / day of second count}) + \dots + (\text{Number of germinated seeds at last count / day of last count}).$$

Subsequently, the emergence of the seedlings (as a percentage of the seeds) was also recorded in a cell tray experiment in a glasshouse. Minimum/maximum air temperature and relative humidity were: 22/38 °C and 36/54 %, respectively and the seeds and seedlings were subjected to a natural day length ranging between 12 and 14 h during the experiment. The seeds were primed with GA_3 , KNO_3 , and PEG as previously described and seeds were sown and covered with soil in cell trays (20 cells for each treatment). It was used a slightly calcareous sandy clay loam (SCL) soil ($pH = 7.1$). Cell dimensions were $1\frac{3}{4}'' \times 1\frac{3}{4}''$ and seed tray outer dimensions were $21\frac{1}{4}'' \times 11\frac{1}{4}'' \times 2\frac{1}{4}''$. The plants were irrigated with equal amount of water (5 ml in each cell) every day and seedling emergence was determined. After 30 days, seedlings were cut in the soil surface, their length was measured and the dry weight was also recorded after oven drying at 60 °C for 24 h.

The percentages of germination and emergence (after arcsine transformation) were subjected to one-way analysis of variance (ANOVA) using the Statgraphics statistical software package (v.5.0, Statistical Graphics Corporation, Englewood Cliffs, NJ, USA). Mean comparison was performed using Fisher's least significant difference (LSD) method ($P = 0.05$).

RESULTS

The effects of the several seed priming treatments on germination percentage and germination rate are presented in Table 1. GA_3 at 400 ppm, potassium nitrate (at both concentrations) and PEG significantly increased germination percentage of *A. millefolium*. Germination rate was also significantly increased as a result of all seed priming techniques.

In Table 2, the effects of seed priming on seedling emergence percentage and germination rate of *A. millefolium* is shown. Seedling emergence was significantly increased by GA_3 at 400 ppm, potassium nitrate (at both concentrations) and PEG compared with the untreated seeds. Germination rate was also positively and significantly affected by the same seed treatments, while the effect of GA_3 at 800 ppm and KNO_3 (2%) was no significant ($p > 0.05$).

Greenhouse experiments also revealed significant differences in the shoot length and dry weight of *A. millefolium* plants between the several treatments (Table 3). In particular, GA_3 treatments resulted in the highest plants. In addition, dry biomass was significantly enhanced by means of GA_3 (at 400 and 800 ppm), KNO_3 (4%) and PEG for 24 h.

DISCUSSION

The present study was aimed at determining the potential effects of several seed priming methods on seed germination and seedling emergence and growth parameters of *A. millefolium*.

Table 1: Germination percentage and germination rate of *A. millefolium* in response to several seed priming treatments (experiment in Petri dishes). Means within a column followed by the same letter are not significantly different according to Fischer's least significant difference test at a $P=0.05$ level

	Germination percentage (%)	Germination rate
Untreated	58±2.6 ^a	22±0.4 ^a
GA_3 400 ppm	67±0.3 ^b	29±0.8 ^b
GA_3 800 ppm	57±1.8 ^a	27±0.6 ^b
KNO_3 (2%)	64±2.2 ^{a,b}	29±0.5 ^b
KNO_3 (4%)	71±1.7 ^{b,c}	32±0.4 ^b
PEG (12 h)	77±2.8 ^{c,d}	38±0.9 ^c
PEG (24 h)	84±2.6 ^d	36±0.5 ^c

Table 2: Seedling emergence percentage and germination rate of *A. millefolium* in response to several seed priming treatments (experiment in soil). Means within a column followed by the same letter are not significantly different according to Fischer's least significant difference test at a $P=0.05$ level

	Seedling emergence (%)	Germination rate
Untreated	55±1.1 ^a	23±0.3 ^a
GA_3 400 ppm	63±0.5 ^b	31±0.4 ^b
GA_3 800 ppm	56±1.2 ^a	25±0.5 ^a
KNO_3 (2%)	61±1.3 ^{a,b}	28±0.4 ^{a,b}
KNO_3 (4%)	67±1.2 ^b	30±1.3 ^b
PEG (12 h)	74±2.1 ^c	33±0.6 ^{b,c}
PEG (24 h)	78±1.7 ^c	35±1.5 ^c

Table 3: Shoot length and above-ground dry weight of *A. millefolium* seedlings in response to several seed priming treatments (experiment in soil). Means within a column followed by the same letter are not significantly different according to Fischer's least significant difference test at a P=0.05 level

	Shoot length (cm)	Above-ground dry weight (g)
Untreated	16.1±1.6 ^a	4.3±0.3 ^a
GA ₃ 400 ppm	22.2±1.9 ^c	5.1±0.7 ^{b,c}
GA ₃ 800 ppm	23.3±2.3 ^c	5.3±0.8 ^c
KNO ₃ (2%)	16.6±0.9 ^a	4.4±0.6 ^a
KNO ₃ (4%)	17.2±1.6 ^{a,b}	5.1±0.6 ^{b,c}
PEG (12 h)	19.4±0.9 ^b	4.5±0.3 ^a
PEG (24 h)	17.2±1.3 ^{a,b}	4.9±0.4 ^b

Our results on seed germination are in accordance with Mirshekari *et al.* (2013), who found that germination percentage of untreated *A. millefolium* seeds was not higher than 70% [16]. Fetri *et al.* (2014) studied the effects of salinity and drought on *A. millefolium* germination and seedling growth and revealed that drought and salinity stresses reduced significantly germination rate, germination percentage and shoot length [19].

Due to the low and variable seed germination, any practices that may enhance germination and emergence of *A. millefolium* are urgently needed [15]. Seed quality enhancement techniques improve seed germination of medicinal and aromatic crops. Hoseini *et al.* (2013) reported that seed priming treatments in two varieties of lemon balm increase antioxidant enzymes and germination percentage [20]. Several seed priming techniques have been used on *A. millefolium*, with positive effects in the majority of them [16]. Sedghi *et al.* (2010) reported that seed priming had significant effect on germination percentage, germination speed, root and shoot length in two medical plants (marigold and sweet fennel) [21].

In the present study, seed priming through the use of gibberellin acid at 400 ppm had a positive effect on seed germination and germination rate and this finding is in full agreement with the findings of previous studies, revealing the effects of gibberellin acid on the seed germination of different species [22]. In addition, the improvement of seed emergence and growth characteristics of *A. millefolium* by means of GA₃ has been previously reported in other medicinal species like *Anthemis pseudocotula* [23]. Similarly, in *Calendula officinalis*, Ganji Arjenaki *et al.* (2011) observed that osmo-priming with PEG-6000 enhanced germination percentage, germination rate and radicle and shoot lengths [24]. Furthermore, Fariman *et al.* (2011) also found that seed priming improved germination percentage and speed of germination in *Echinacea purpurea* [25]. Regarding potassium nitrate and its effect on emergence and initial seedling establishment, Tzortzakis (2009) revealed that there were positive effects on weight of *Cichorium* spp. seedlings [26]. This is in agreement with our results and the results of another study on *Borago officinalis*, indicating the importance of seed priming for seed germination and field performance [27].

CONCLUSIONS

The benefits of different seed priming techniques in *A. millefolium* include higher and faster seed germination and seedling emergence and highest early growth and biomass production. In particular, GA₃ at 400 ppm, potassium nitrate (at both concentrations) and PEG significantly increased germination percentage of *A. millefolium*, while germination rate was also significantly increased as a result of all seed priming techniques. Seedling emergence was significantly increased by GA₃ at 400 ppm, potassium nitrate (at both concentrations) and PEG compared with the untreated seeds. Dry biomass of the young seedlings was significantly enhanced by means of GA₃ (at 400 and 800 ppm), KNO₃ (4%) and PEG for 24 h, indicating the potential effect of seed priming on first growth as well.

AUTHOR'S CONTRIBUTORS

All the authors contributed equally in the paper. PK, VD and IK have carried out the experiments and interpreted the data. PK and PP have designed the experiment and validated the manuscript. Similarly, PK and PP wrote the manuscript.

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