Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 5 (4): 695-699, 2006
ISSN 1682-3974
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Breaking Seed Dormancy in *Bunium persicum* by Stratification and Chemical Substances

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Abstract: Ripe fruits of *Bunium persicum* are reported to contain an essential oil rich in monoterpene aldehydes. This spice is not cultivated in Iran because of seed dormancy and is found only in some natural habitats. This study was conducted to identify effective treatments for breaking dormancy in *B. persicum* seeds. Growth regulators, osmotic priming, nitrogenous compounds and cold stratification were tested. Benzyladenin and kinetin achieved dormancy breaking in *B. persicum* seeds. When the seeds were exposed to benzyladenin in combination with polyethyleneglycol and gibberellic acid the germination percentage was improved. Potassium nitrate, thiourea, polyethyleneglycol and NaEDTA (when applied alone) were ineffective. The seed germination was increased by cold stratification, where by increasing the duration of stratification from four, six, eight to ten weeks the germination percentage was increased, respectively. Combination of the stratification with benzyladenin treatment had promoted effect on germination. It is concluded that the above mentioned effective treatments could be used to cultivate the spice in order to obtain its essential oil.

Key words: *Bunium persicum*, seed dormancy, seed germination

INTRODUCTION

The umbelliferae contains about 3000 species of plants dispersed throughout the world, especially in the North hemisphere (Baskin *et al*., 1992). An important problem often encountered in the culture of members of the umbelliferae has been poor germination because of seed dormancy (Robinson, 1954). The fruits of *Bunium persicum* (Boiss.) Fedtsch which grows wild in the middle East, particularly in Iran, are rich in essential oil but its seed exhibit poor germination as a result of dormancy.

To break dormancy in seeds, different methods such as followings are used depending on the type of dormancy and plant species (ISTA, 1993). Exogenous hormones are reported to influence seed dormancy and germination. Virtually all processes connected with growth, development and metabolism in plants and animals are governed in one way or another by hormones (Khan, 1971; Chuanren *et al*., 2004). It is suggested that the onset of embryo dormancy is associated with accumulation of growth inhibitors and breaking of dormancy with a shift in the balance of growth regulators towards growth promoters that overcome the effect of inhibitor (Khan, 1971).

Individual hormones in a seed, at any one time, are at physiologically effective or a physiologically ineffective concentration. The effective or ineffective concentrations of hormones in a biological system, such as a seed, in turn, must depend on many metabolic and environmental factors (Khan, 1971). Abscisic acid (ABA) and gibberellins (GAs) are the hormones proposed to control primary dormancy, ABA by inhibiting and GAs by inducing germination (Hilhorst and Karssen, 1992; Iglesias and Babiano, 1997).

Some nitrogenous compounds, such as nitrate, nitrite and thiourea, are known to stimulate the germination of seeds (Yoshiyama *et al*., 1996). Nitrate often enhances the action of growth regulators such as gibberellins, cytokinins and ethylene. Thiourea counteracts the effect of ABA and reduces the level of cytokinins in plant tissues (Kabar and Baltepe, 1989). Osmotic agents can also have a positive effect on seed germination (Bradford, 1986; Bewely and Black, 1994). Polyethyleneglycol (PEG) has been used for many years in osmotic priming, the seeds exposed to PEG imbibe sufficient water to initiate the germination process, but radical emergence is prevented by the osmotic potential of the solution (Bradford, 1986).

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Moist chilling or cold stratification has been widely used as a pre-sowing treatment for breaking dormancy and enhancing the maximum rate and percentage germination of dormant seeds of many species (Baskin *et al*., 2001; Karam and Al-Salem, 2001). The technique is simple, inexpensive and effective in overcoming seed dormancy, although the phenomenon is not yet fully explained. However, the effects of moist chilling in establishing hormonal levels that favor germination have been suggested to result from cold-stimulation of appropriate enzyme activity (Nikolaeva, 1997). The objective of this study were to determine the effect of growth regulators, osmotic priming, nitrogenous compounds and cold stratification on seed germination and to advise an effective method for breaking seed dormancy of *B. persicum*.

MATERIALS AND METHODS

Seeds of *B. persicum* were harvested in the Kerman mountains in Iran. After seed sterilization and determination of viability with tetrazolium method (Karam and Al-Salem, 2001), several experiments were conducted to determine the effects of different methods on seed dormancy breaking and subsequent germination.

In the primary experiments, the seeds were treated with GA_3 (gibberellic acid) [10 or 100 mg L^{-1}], BA (benzyladenin) [10^{-4} or 10^{-5} M], Kin (kinetin) [10^{-4} or 10^{-5} M], KNO_3 [2, 4 or 6 g L^{-1}], PEG (polyethylenglycol) [30 g L^{-1}] and thiourea (1, 5, 10 or 50 g L^{-1}). In all experiments a control treatment was set up using distilled water.

In the second set of experiments, the effects of combined application of GA_3 (10 or 100 mg dm^{-3}), PEG (30 g dm^{-3}) or NaEDTA (10^{-3} M) with BA (10^{-5} M) on seed germination were examined. For determination of the interaction of NaEDTA or PEG with BA, seeds were soaked in NaEDTA or PEG solutions and shaken on orbital shaker for two days. Seeds were then removed, placed on a filter paper moistened with BA or distilled water as a control. For combined exposure of GA_3 and BA, seeds were placed on a filter paper moistened with BA plus GA_3 or distilled water as control.

In the third set of experiments, for cold moist stratification, seeds were placed between two layers of filter paper in moist perlite, in 15 cm diameter glass Petri dishes and placed at 4°C for 1, 2, 3, 4, 6, 8 and 10 weeks. After this period the seeds of each treatment were placed on filter paper moistened with distillated water, for germination. The controls were not exposed to 4°C.

In another experiment, effects of BA, GA_3 or Kin treatments on germination of stratified seeds were studied. In this step, first the seeds were incubated at 4°C for four weeks, then the seeds were placed on filter paper moistened with BA (10^{-5} M), GA_3 (10 or 100 mg dm^{-3}), Kin (10^{-5} M) or distilled water as control.

In all experiments, germination was carried out in 8 cm plastic petri dishes. Four replicates of 25 seeds were used in each treatment. All dishes were sealed with a strip of parafilm to reduce water loss. Darkness was maintained by wrapping the dishes with two layers of aluminum foil. Dishes were placed at 15°C and germinated seeds were counted every week for four weeks. Seeds were considered germinated when the radicle emerged. The significance of treatments means was tested by one-way Analysis of Variance using SPSS for Windows and Duncan's test was used for means comparisons ($p<0.05$).

RESULTS

This study showed that germination percentage of non-treated seeds at 15°C in dark was very low after thirty days. In the primary experiments, only treatments involving BA (10^{-5} M) and Kin (10^{-5} M) significantly improved germination where 68.7 and 48.4% of the seeds were germinated in these treatment, respectively (Table 1). As indicated in Table 1, no significant difference was found among the other treatments.

Positive effect of BA(10^{-5} M) on seed germination was increased when it co-applied either with GA_3 (10 mg L^{-1}) or with PEG and higher germination was achieved, 81.2 and 73.4%, respectively (Table 2). In contrast, when BA(10^{-5} M) was co-applied with NaEDTA, the germination percentage decreased to 49.9% compared to 68.7% of BA(10^{-5} M). As depicted in Table 3, germination percentage of *B. persicum* seeds was not affected by stratification at 4°C for 1, 2, 3 weeks. In contrast, in the presence of stratification for 4, 6, 8 and 10 weeks, the germination percentage was 24.9, 45.27, 58.40 and 57.60%, respectively.

The effects of some of the above mentioned chemical treatments on the seed germination when the seeds were stratified are shown in Table 4. In presence of stratification for 30 days, only 24.9% of seeds germinated. When the stratified seeds were treated with BA(10^{-5} M), Kin(10^{-5} M) or GA(100 mg L^{-1}) the germination percentage increased to 92.2, 45.3 and 41.6, respectively. Comparison of the results shown in Table 1 and 4

Table 1: Final germination percentage of *Bunium persicum* seeds in response to different treatments (Means±SE) after 4 weeks

Treatment	Germination (%)
Control	1.50±1.5a
GA_3 (10 mg L^{-1})	3.60±1.8a
GA_3 (100 mg L^{-1})	0.0a
Kin (10^{-4} M)	6.20±2.5a
Kin (10^{-5} M)	48.40±5.3b
BA (10^{-4} M)	12.50±4.4a
BA (10^{-5} M)	68.70±10.2c
PEG	1.56±1.56a
NaEDTA	1.56±1.56a
KNO_3	0.0a
Thiourea (50 g L^{-1})	7.80±2a
Thiourea (1, 5, 10 g L^{-1})	0.0a

The mean followed by the same letter(s) are not significantly different ($p<0.05$)

Table 2: Germination of *Bunium persicum* seeds in response to combined application of GA_3 or PEG or NaEDTA with BA after 4 weeks

Treatment	Germination (%)
Control	1.5±1.5a
NaEDTA + BA(10^{-5} M)	49.9±6.2b
PEG + BA(10^{-5} M)	73.4±5.3c
GA_3 (10 mg L^{-1}) + BA(10^{-5} M)	81.2±6.7c
GA_3 (100 mg L^{-1}) + BA(10^{-5} M)	6.2±0.7a

The means followed by the same letter(s) are not significantly different ($p<0.05$)

Table 3: Final germination percentage of *Bunium persicum* seeds in response to duration of stratification at 4°C after 4 weeks

Stratification duration (week)	Germination (%)
0, 1, 2, 3	0.0a
4	24.90±6.7b
6	45.27±3.9c
8	58.40±7.7c
10	57.60±8c

The means followed by the same letter(s) are not significantly different ($p<0.05$)

Table 4: Germination of stratified *Bunium persicum* seeds in response to different treatments after thirty days of cold treatment

Treatment	Germination (%)
Control	24.9±6.7a
BA (10^{-5} M)	92.2±11.1b
Kin (10^{-5} M)	45.3±6.9ab
GA_3 (10 mg L^{-1})	15.6±1.8a
GA_3 (100 mg L^{-1})	41.6±2.1a

The means followed by the same letter(s) are not significantly different ($p<0.05$)

represented that BA and GA_3 are more effective on stratified seed than on non-stratified, where in presence of stratification and BA, 92.2% of the seeds were germinated.

DISCUSSION

The results of this study demonstrated the effect of cytokinins (BA and Kin) on dormancy breaking in seeds of *B. persicum*. Present findings were confirmed by a number of researchers who showed similar effects of cytokinins in germination (Overbeek *et al*., 1967; Aberlenc-Bertossi, 1999; Naidu and Rajendrudn, 2001; Parks and Boyle, 2002). The cytokinins probably penetrates the testa and neutralizes the inhibitors present in the embryo, thus enabling the embryo to rupture the seed coats (Khan, 1971). In the present study, the combination of BA with other promotive agents such as GA_3, PEG or moist chilling was more efficient on dormancy breaking of the seeds. It has been, also, reported that seed germination is promoted by using GA_3 or BA in many plant species. GA is an important endogenous growth regulator that has profound and diverse effects on plant growth and development, in contrast cytokinins although not affecting germination directly, appears to be essential for completion of gibberellin-induced germinative processes (Khan, 1971). According to the amplification of stimulatory effects of GA_3 and BA in this study, conclusion can be drawn that GAs are permitted to reach their active sites through the modifying influence of cytokinins on transport across membranes andare thus able to initiate the biochemical processes necessary for germination to occur (Thomas *et al*., 1975).

Schmitz *et al*. (2001) found that PEG causes a change in water relations of the seeds. When PEG is removed, water uptake is rapid and the first phase of germination, imbibition, occurs better so that germination percentage was increased.

Present results also showed, stratification at 4°C was very successful in breaking dormancy of seeds and increasing the duration of stratification resulted in an increase in germination percentage. Present findings were confirmed with many researches (Bungard *et al*., 1997; Wang and Berjak, 2000; Rehman and Park, 2000; Baskin *et al*., 2001; Greipsson, 2001; Karam and Al-Salem, 2001; Parks and Boyle, 2002). Moist chilling is a standard procedure used to enhance the germination of dormant seeds. It has been used for various dormant seeds and has been reported successfully to alleviate endogenous dormancy. There are conflicting results in the literature about the required length of the period of cold-moist treatment, which varied from 2 to 15 weeks. In this study, 8 weeks chilling get the maximum germination percentage.

It is believed that cold treatment can only work for those seeds that contain both inhibitors and promoters as evidenced by the fact that the inhibitor-promoter balance is altered by exposing seed to moist chilling (Frankland and Wareing, 1962). On the other hand, increased germination in the stratified seeds subjected to plant regulators suggested that interplay of several hormones may be required at times for the completion of germinative processes. Thus, phytohormones might have designated functions in the control of germination and dormancy with GA assuming the primary role and inhibitors and cytokinins assuming the preventive and permissive roles, respectively.

Nitrogenous compound such as nitrate and thiourea are reported to alleviate dormancy in seeds (Gul and Weber, 1998; Khan and Ungar, 2001; Plummer *et al*., 2001) but were unable to alleviate dormancy in seeds of *B. persicum*. Applied concentrations of NaEDTA were ineffective in the absence of BA but stimulated germination when BA was present. This results are consistent with the results of Thomas *et al*. (1975) that reported NaEDTA only in presence of $GA_{4/7}$ could stimulate germination.

According to the results of this study, since plant regulators such as BA or stratification treatments breaks dormancy in *B. persicum* seeds it could be concluded that inability to germinate in *B. persicum* is a complicated process that is controlled by both external and internal regulating factors. The results suggest that co-application of BA and moist chilling is the most effective treatment to break dormancy in *B. persicum* seeds.

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