In Vivo Propagation of Akub (Gundelia tournefortii L.) by Seeds
(RESEARCH NOTE)
Rida A. Shibli*, Dana S. Oweis**, Khalil I. Erfiefej** and Mohamad A. Shatnawi**

ABSTRACT
Two experiments, one in the laboratory and one in the greenhouse, were conducted to study the propagation of Gundelia tournefortii by seeds. In the lab experiment, different concentrations of GA$_3$ (0.0, 100, 300 and 500 ppm), KNO$_3$ (0.1, 0.2 and 0.3% (w/v)) and thiourea at (1, 2 and 3% (w/v)) were studied to break seed dormancy of Gundelia tournefortii. In the greenhouse experiment, the effects of different concentrations of GA$_3$ (0.0, 250, 500 and 750 ppm) and different soaking durations (6, 12, 24 and 48 hours) on seed germination of Gundelia tournefortii were investigated. Using 100 and 300 ppm GA$_3$ gave the highest germination percentage (50% and 63%, respectively) in the lab experiment, whereas the 500 ppm GA$_3$ concentration reduced seed germination. In the greenhouse experiment, soaking the seeds in 250 ppm GA$_3$ for 6 hours resulted in the highest germination percentage (83%).

Keywords: Propagation, Akub (Gundelia tournefortii L.).

INTRODUCTION
Gundelia tournefontii L. or Akub as known in colloquial Arabic (English common name: tumble thistle) is a spiny perennial herb thistle which belongs to the compositae family, found in the plains and mountains of the Mediterranean region (Oweis et al., 2004). Gundelia tournefortii L. is consumed as a fresh or cooked artichoke-like vegetable. The edible parts of G. tournefontii (stem and head) have high nutritive value (22% protein, 18% fiber, 0.04% Fe and 0.45% Ca) (Oweis, 2003). The plant prefers light sandy or medium loam soils and requires well-drained soil. Gundelia grows in a wide range of soil pH, cannot grow in the shade and requires moist soil (Post and Dinsmore, 1933; Zohary, 1962; Oweis et al., 2004). G. tournefortii plants have thick perennial rootstocks from which new growths arise each season. Depending on the rainfall and temperature profile of the season, the plants develop new rosettes (Lev-Yadun and Abbo, 1999). The presence of Gundelia tournefortii L. in Jordan is threatened by land cultivation and overgrazing (Oweis et al., 2004).

Regeneration from seeds is the most often used and cheapest method of propagation in several plants (MacDonald, 1993; Willenborg et al., 2005). Seeds are produced in large numbers, easily stored for long periods with less loss of viability, easily distributed and can produce plants free of most of the pests and diseases (George, 1993; Willenborg et al., 2005; Mos’ et al., 2006; Al-Karaki et al., 2007). Dormancy can be defined as the lack of seed germination, due to unfavorable
environmental conditions (external dormancy) or to factors within the seed (internal dormancy) (Desai et al., 1997; Hartmann et al., 1997; Tylorson and Hendricks, 1977; Willenborg et al., 2005; Mos' et al., 2006; Al-Karaki et al., 2007). Seed dormancy can occur due to various factors. It may be due to the immaturity of the embryo, impermeability of seed coat to water or gases, prevention of embryo development due to mechanical causes, special requirements of temperature or light or the presence of substances inhibiting germination (Nikolaeva, 1969; Duffus and Slaughter, 1980; Willenborg et al., 2005; Mos' et al., 2006; Al-Karaki et al., 2007). There are various methods that have been used to break seed dormancy in different kinds of seeds which include scarification (Hartmann et al., 1997; Oweis et al., 2004; Willenborg et al., 2005; Mos' et al., 2006) and stratification (Agrawal and Dadlani, 1995). Also, various chemical substances can completely or partially substitute for light or temperature in breaking seed dormancy. These substances vary in chemical nature. Some of them are simple compounds, such as potassium nitrate and thiourea, while others are complicated molecules such as the hormones (gibberellins and cytokinins) (Mayer and Mayber, 1982).

This study was initiated to develop a protocol for propagation of *G. tournefortii* by seeds. Factors affecting seed germinating were studied in the laboratory and in the greenhouse.

**MATERIALS AND METHODS**

**Seed Viability Testing**

Seeds of *Gundelia tournefortii* used in this investigation were collected from the northern region of Jordan, Ajloun Mountain 35° 36´-35° 54´ East longitudes, 32° 11´-32° 24´ North latitudes, 960 m above sea level altitude and with 574 mm average annual rainfall. Seeds were extracted from the dried inflorescences and kept at 4 °C in nylon bags until used in the experiments. Initial estimates of seed viability were determined for four representative samples (replicates) of 20 seeds each using the tetrazolium method (International Seed Testing Association, 1993). A 1 % solution of 2, 3, 4-triphenyl tetrazolium chloride (TTC) was used.

**Laboratory Experiment**

The effect of different chemical treatments on breaking seed dormancy and germination was studied in the lab. Seeds were placed between two layers of filter paper moistened with 10 ml of different treatments in 9 cm plastic Petri dishes for 20 min. Treatments were GA$_3$ [0, 100, 300 and 500 mg/L], KNO$_3$ [0.1, 0.2 and 0.3 % (w/v)] and thiourea [1, 2 and 3 % (w/v)]. Seeds were then placed in an incubator, maintained in dark at 24 ± 2 °C and moistened as needed with distilled water. For each treatment, there were three replicates per treatment arranged in a completely randomized design (CRD). For each replicate, 10 seeds were placed in a Petri dish. Seeds were considered germinated when radicails emerged and reached around 5 mm.

**Greenhouse Experiment**

In the greenhouse experiment, dry seeds were soaked in GA$_3$ at 250, 500 and 750 mg/L for 6 hours and sown in 1 Peat : 1 Perlite mixture in polystyrene trays (20 seeds / treatment) under intermittent mist. Treatments were arranged in a completely randomized design (CRD) with five replicates per treatment. Germination percentage was recorded after four weeks. In another experiment, seeds were soaked in 250 mg/L GA$_3$ for 6, 12, 24 and 48 hours and then were sown in 1 Peat: 1 Perlite mixture in polystyrene trays. Treatments were arranged in a completely randomized design with five replicates per treatment (each replicate consisted of 20 seeds). Germination percentage was recorded after four weeks.
Statistical Analysis

Data in each experiment were subjected to the analysis of variance (ANOVA) and means were separated according to the least significant difference (LSD) at 0.05 level of probability (SAS, 1989).

RESULTS AND DISCUSSION

Seed Viability Testing

Viability percentage of *G. tournefortii* seeds as estimated by TTC was 90%. This indicates that seeds would be capable to germinate under favorable conditions and that failure of germination would not be attributed to unviable seeds, but mainly due to seed dormancy. The high viability percentage resulted from the fact that these seeds were fresh and not stored before performing the tetrazolium test (Al-Bukhari, 1997). Tetrazolium chloride method has gained popularity mainly because of its simplicity, ease and rapidity of application (Pant et al., 1999).

Laboratory Experiment

Significant differences in seed germination of *G. tournefortii* among GA$_3$, KNO$_3$ and thiourea treatments were obtained (Fig. 1). Using 100 or 300 ppm of GA$_3$ resulted in the highest germination percentage (50% and 63%, respectively). Using 500 ppm of GA$_3$ reduced seed germination as compared with 100 or 300 ppm. Soaking seeds in 0 ppm GA$_3$ (pure water) produced the lowest germination percentage (33%). Similarly, Karam and Al-Salem (2001) reported that the treatment of *Arbutus andrachne* with GA$_3$ was successful in breaking seed dormancy, where 250 ppm resulted in 86% germination and higher concentrations decreased germination. Also, the 250 ppm GA$_3$ significantly enhanced seed germination (43%) of *Aconitum balfourii* but this was inhibitory for *Aconitum heterophyllum* (Pandey et al., 2000). On the other hand, there was no significant difference between KNO$_3$ and thiourea on seed germination (Fig. 1). Gebre (2002) reported that KNO$_3$ was not effective in enhancing seed germination of *Cercia silquastrum* and that thiourea inhibited seed germination. Similarly, KNO$_3$ was not effective in enhancing germination of *Arbutus andrachne* seeds and thiourea failed to break seed dormancy (Karam and Al-Salem, 2001). Pandey et al. (1999) showed that the nitrogenous compound (KNO$_3$) enhanced seed germination (80%) of *Aconitum balfourii*, while in *Aconitum heterophyllum* only thiourea was effective. The stimulation obtained by KNO$_3$ is dependent on its concentration (Mayer and Mayber, 1982).

Greenhouse Experiment

A highly significant effect of GA$_3$ on seed germination of *G. tournefortii* is shown in Figure (2). The highest seed germination percentage was at 250 ppm of GA$_3$ (83%). There was no significant difference in seed germination between dry seeds and seeds soaked in water (57 and 53 %, respectively). Using 750 ppm of GA$_3$ resulted in complete inhibition of seed germination. Badoni (1994) demonstrated that using 100 ppm of GA$_3$ enhanced seed germination of *Aconitum atroxb*, *Aconitum heterophyllum*, *Sussurea lappa* and *Selinum wallichianum*. However, in *Rheum australe* GA$_3$ treatment did not show any positive effect.
Figure (1): Germination percentage of *G. tournefortii* seeds as influenced by different concentrations of GA$_3$, KNO$_3$ and thiourea in the lab experiment. Bars represent LSD values at 0.05 level of probability.

Figure (2): Germination percentage of *G. tournefortii* seeds as influenced by different concentrations of GA$_3$ in the greenhouse experiment. Bars represent LSD values at 0.05 level of probability.
A significant effect of soaking duration of *G. tournefortii* seeds in GA\textsubscript{3} on seed germination was reported (Fig. 3). Germination percentage increased drastically (up to 87\%) when seeds were soaked in 500 ppm of GA\textsubscript{3} for 6 hours. Seeds soaked in 250 ppm of GA\textsubscript{3} for 48 hours showed a very low germination percentage (7 \%). Rania et al. (2000) reported that seeds of *Gloriosa superba* treated with 50 ppm of GA\textsubscript{3} for 6 hours resulted in 60\% germination.

In conclusion, this study presented for the first time a germination protocol for *G. tournefortii*. Seeds are considered good starting material for *in vivo* culture establishment due to their high viability and germination percentage. Soaking *G. tournefortii* seeds in 250 ppm GA\textsubscript{3} for 6 hours was the most successful treatment for breaking seed dormancy.

**REFERENCES**


Dehradun, U.P. Total outlay: Rs. 3, 40, 325.
In Vivo Propagation... Rida A. Shibli, Dana S. Oweis, Khalil I. Eriefej and Mohamad A. Shatnawi

***ﺍﻟﺒﺫﻭﺭ ﺑﻭﺍﺴﻁﺔ ﺍﻟﻌﻜﻭﺏ ﺍﻜﺜﺎﺭ ﻋﻠﻤﻴﺔ ﻓﻲ ﺍﻟﺘﺅﺜﺭ ﺍﻟﺘﻲ ﺍﻟﻌﻭﺍﻤل ﺑﻌﺽ ﺗﻤﺕ (Gundelia tournefortii) ﺍﻟﺘﺨﺭﺒﻴﺔ ﻭﺠﺭﻴﺘ ﻋﺩﺓ ﺗﺩﺭﺎﺴﺔ ﻤﺘ.. 500,300,100,0 ﺍﻟﺠﺒﺭﺘﻤﺕ ﻋﺭﻡ ﻣﺴﺘﻭﻯ ﺑﺎﻟﻤﻠﻴﻭﻥ ﺟﺯﺀ ﺑﺘﺭﺎﻜﻴﺯ ﺍﻟﺒﻭﺘﺎﺴﻴﻭﻡ ﻭﻨﺘﺭﺍﺕ ﻋﻭﺍﻤﻟﺎً ﺑﻌﺽ. 0.1, 0.2, 0.3 % ﺍﻟﺜﻴﻭ ﺍﺭﻴﻴﻭﻤﺎﺩﺓ ﻓﻲ ﻣﺴﺘﻭﻯ 1, 2, 3 % ﻋﺎﻟﻴﺔ ﺍﻟﺘﺭﺍﻜﻴﺯ ﻝﻙ ﻣﺴﺘﺭ ﺍﻟﺘﻘﻠﻴل ﻋﺩٍ ﺍﻟﻨﺘﺎﺌﺞ ﻓﻘﺩ؛ ﺍﻟﺴﺘﺨﺩﺍﻤﻉ ﺑﺎﻟﻤﻠﻴﻭﻥ ﺟﺯﺀ (Gundelia tournefortii) ﺍﻟﺠﺒﺭ ﻋﺭﻡ ﻣﺴﺘﻭﻯ ﺑﻨﺴﺒﺔ ﻣﺴﺘﺭ ﺍﻟﻨﺴﺏ ﻋﺭﻡ ﻋﻨﺩ ﻋﺪﺓ ﺗﺩﺭﺎﺴﺎً ﻭﻋﻨﺩ ﺍﻟﺨﺘﻠﻔﺎً ﻋﺯﺍﺀ ﺟﺯﺀ ﺑﺎﻟﻤﻠﻴﻭﻥ ﺟﺯﺀ (Gundelia tournefortii) ﺍﻟﺠﺒﺭ ﻋﺭﻡ ﻋﺩﺓ ﺑﺎﻟﻤﻠﻴﻭﻥ ﺟﺯﺀ ﻋﻨﺩ ﻋﺪﺓ ﺗﺩﺭﺎﺴﺎً ﻭﻋﻨﺩ ﺍﻟﺨﺘﻠﻔﺎً 750,500,250 ﺑﺎﻟﻤﻠﻴﻭﻥ ﺟﺯﺀ ﻋﻨﺩ ﺍﻟﺨﺘﻠﻔﺎً ﻋﺯﺍﺀ ﺟﺯﺀ ﺑﺎﻟﻤﻠﻴﻭﻥ ﻋﺩﺓ ﺑﺎﻟﻤﻠﻴﻭﻥ ﺟﺯﺀ ﻋﻨﺩ ﺍﻟﺨﺘﻠﻔﺎً 

(Electronic mail: r.shibli@ju.edu.jo)

300,000.0 ﺍﻟﺠﺒﺭ ﻋﺭﻡ ﻃ.secret совместно с пресс-службой Кыргызстана. Об этом говорится в сообщении, опубликованном на сайте президента Кыргызстана. В сообщении отмечается, что в связи с обострением ситуации в регионе было принято решение о введении особого режима в стране. Согласно этому решению, в Кыргызстане будет введен режим самоизоляции, который将持续 до 27 июля. Ожидается, что эта мера поможет снизить количество заражений коронавирусом. В сообщении также отмечается, что Правительство страны будет продолжать принимать все необходимые меры для предотвращения распространения пандемии. Важно отметить, что все граждане страны должны быть информированы о мерах безопасности и соблюдать все рекомендации органов здравоохранения. Всем просьба следить за новостями и выполнять все установленные правила по отношению к коронавирусу.