Genetics of Thebaine and Innovative Seed Germination Techniques in Papaver Bracteatum

Dr.M.Phani Raja kumar[1]
TIA Research Centre, University of Tasmania, Hobart, Australia

ABSTRACT : The potential of Papaver bracteatum as an economical source of Thebaine, an alkaloid used for the production of opiates and other medicinally important compounds has been the catalyst for worldwide research on the species (WHO, 1980). Tasmania, Australia, is the only region in the southern hemisphere currently permitted to grow poppies for the licit medical opiates. Commercial interest from pharmaceutical companies on the medicinal value of P. bracteatum has led to its evaluation as a new crop in Tasmania.

Indexterms: Seed germination, Temperature, Water Potential, Crop Genetics, Light requirement

1. Introduction

The planting material used to establish preliminary field trials in Tasmania displayed characteristics typical of wild species, with uneven seedling emergence and low stand densities recorded (DPIWE, 2002). To overcome these challenges a greater understanding of seed dormancy and germination requirements is required. Ideally, crop establishment from seed will lead to even emergence of plants with uniform spacing between plants at the target density.

Seed germination is a process that commences with water uptake by the seeds and ends with the emergence of the radicle (Bewley, 1997; Bewley and Black, 1994). Temperature and water availability during imbibition have been widely reported to be the main factors that determine the level and rate of germination of non-dormant seeds in laboratory tests and in field trials (Benech-Arnold and Sanchez, 1995). Under optimal moisture conditions, germination begins with rapid uptake of water driven primarily by seed matric potential. Small changes in Ψm (matric potential) have been shown to influence seed water uptake and germination rate to a much greater extent than changes in osmotic potential (Ψs) (Hadas and Russo, 1974). Water stress during germination may decrease or delay seedling emergence, reduce plant growth rate and over the growing season reduce crop biomass (Garwood, 1979; Huang, 1997). Sensitivity of small seeds to water stress during germination is particularly high because of limited availability of carbohydrate reserves (Billings, 1976). The range of temperatures over which a seed lot germinates is primarily dependant on the species, but is also influenced by seed lot quality and the degree of dormancy (Bewley, 1997).

In order to predict the performance of seed-lots in a cropping system, it is recommended that seed lots be assessed in terms of the degree of dormancy, level of viable seeds, uniformity of germination and capacity to germinate across a broad range of sub or supra-optimal conditions (Copeland and McDonald, 1995). In addition, the uniformity of germination and the time needed to reach maximum germination percentage also varies with temperature and water availability, and an understanding of these effects has led to the development of hydrothermal models to predict seed-lot performance (Gummerson, 1986). The capacity to predict germination at any temperature and water potential has the potential to direct management of establishment practices in order to optimise crop stands in the field.

Only one study has been published on the seed germination of P. bracteatum. In their study, Bare et al. (1978) investigated the seed germination characteristics of three Papaver species; P. bracteatum, P. orientale and P. somniferum under different light and temperature regimes. The maximum germination level of P. bracteatum seed was achieved at temperatures ranging from 18º to 26ºC and germination rate occurred at temperatures ranging from 21º to 26ºC. This was a narrower optimal temperature range than that recorded for P. orientale and P. somniferum, with the maximum germination rate recorded for the two species ranging from 18º to 30ºC and 13º to 33ºC respectively. In the same study Bare et al. (1978) reported no

germination above 30ºC and when dormancy was induced in P. bracteatum seeds germinated when exposed to 35ºC pre-treatment far red light and red light promoted germination to the same level. The same response was not recorded at lower temperatures with seed able to germinate under both light and dark conditions. In Papaver rhoeas the degree of dormancy decreases during warm periods and increases during cool periods (Baskin et al., 2002; Cirujeda et al., 2006; Karlsson and Milberg, 2007; Milberg and Andersson, 1997). In field conditions, Papaver argemone, Papaver rhoeas, and Papaver dubium were reported to germinate over several seasons irrespective of prevailing climate and weather conditions (Karlsson and Milberg, 2007), suggesting a morpho-physiological dormancy mechanism. The nature and extent of dormancy in P. bracteatum needs to be confirmed as it has the potential to impact significantly on crop establishment from seed. This study addresses the paucity of understanding of the germination requirements of P. bracteatum and investigated the cardinal temperatures for germination, light and water requirements, and the loss in viability during storage.

2.MATERIALS AND METHODS

i) Seed Source

Seed lots were obtained from P. bracteatum plants grown under glasshouse or shade house conditions at the Horticultural Research Centre located at the Sandy Bay campus of the University of Tasmania. Capsules were harvested, dried cleaned as outlined in chapter 2. A series of experiments were undertaken to determine the light, temperature, water potential requirements for germination, and effects of short-term storage on germination.

ii) Light Requirement

Light requirement for the germination of P. bracteatum seed was assessed by comparing three different seed sources ranging from freshly harvested to long-term stored. Seed-lots were denoted A (stored for 26 weeks), B (stored for 52 weeks) and C (freshly harvested). Each seed-lot was imbibed under either continuous light or continuous dark. Four replicates of 50 seed of each treatment (light and dark) were used and were randomly arranged within a controlled temperature cabinet set to 20ºC. Seeds were germinated in petri-dishes sealed with Parafilm (Parafilm® Model 60631, CHICAGO, IL, (101.6 mm) to limit moisture loss. Dark treatment was achieved by individually wrapping petri-dishes with two layers of aluminium foil. The number of germinated seeds was recorded on a daily basis for a period of fourteen days. Seeds germinated in dark conditions were checked under green safe light. When necessary, distilled water was added to petri-dishes during germination assessments to maintain adequate moisture levels.

iii) Effect of Storage

The effect of storage time on germination percentage was assessed using a graded and an ungraded seed-lot, and the rate of loss of viability during storage was calculated from the germination data. The first seed lot, harvested in 2006, was cleaned but not size or density graded and the second seed lot, collected in 2007, was density graded by air-screening (South Dakota seed blower, USA) and size graded with a laboratory sized clipper-cleaner (Blount Agri-Industrial, Indiana, USA). Seed in the median density, median size class was used in the study. Seed lots were stored in double plastic bags at 20ºC for 36 months. Every month, commencing shortly after seed was harvested from the mother plants, a sub-sample of seeds was taken from the bulk seed lot and germinated at 20ºC at 12:12 light in a controlled temperature cabinets. Four replicates of fifty seeds were used in each assessment.

iv) Effect of Temperature

The experiment was conducted to investigate the germination characteristics of P. bracteatum seed lots under a range of constant temperatures. Eight constant temperatures ranging from 5º to 35ºC were obtained on the thermo-gradient table (Refer Chapter 2). Two separate seed lots from the plants grown under glasshouse conditions from two different seasons (2006, stored for 2 years, and 2007, stored for 1 year) were used. Four replicates of 50 seeds from each of the two seed lots were germinated at each temperature. Radicle emergence was assessed every 24 h over a period of 28 days.

3.Temperature and Water Availability

The interaction between temperature and water potential on germination response of P. bracteatum seed was investigated. The experiment consisted of four constant temperatures (9º, 15º, 20º and 30ºC) and three water potentials (0, -0.1, -0.3 MPa). -0.1 and -0.3 MPa water potential solutions were prepared using aqueous solutions of polyethylene glycol (PEG 8000) according to Michel (1983). The ψ- values of the solutions were checked using a vapour pressure osmometer (Model 5100 c; Wescor Inc., Logan, UT, USA), which was calibrated using NaCl standards, and corrected for each constant temperature (Michel and Kaufmann, 1973). Custom built germination cabinets kept on a thermo-gradient table were used. Four replicates of each water potential treatments were randomly allocated to chambers at each temperature. The seeds were placed on the filter paper inside the cabinets and the PEG solution was permitted to wick on to the filter paper, maintaining the seeds at constant water potential. Seeds were transferred every twenty four hours across to fresh chambers to ensure they were exposed to constant water potential in the cabinets over the duration of the experiment. Regular assessment of solution ψ- on filter paper sections using a vapour pressure osmometer confirmed that water potential remained relatively constant for the duration of the experiment. The number of seeds that germinated was recorded daily until 21 days after imbibition.

i)Data analysis

All statistical analyses were performed using SAS version 9.1 (SAS, Institute, Cary North Carolina, USA) statistical package. SAS procedure NLIN was used to describe the time course of germination of individual replicates of fifty seeds using the logistic growth curve equation:

$$Y_t = M\,[1 + \exp(-K*(t - L))]^{-1}$$

Where; Yt is the cumulative percentage germination at time t, M is the asymptote (theoretical maximum for Yt), K is the proportional to the rate of germination, and L is the time to 50% maximum germination, M. The logistic function described above has been widely used in seed germination studies to describe the time course of germination (Dumur et al., 1990; Shafii et al., 1991; Thompson et al., 1994). Data recorded in the light and dark, effect of temperature and effect of water potential experiments were analysed using the GLM procedure to test treatment effects on the maximum germination and time to 50% germination (T-50) consistent with the experimental design. Proc REG was used to determine the relationship between seed viability and duration of storage. Percentage germination data were arcsine square-root transformed to meet the assumptions of normality and homoscedasticity prior to analysis.

4.RESULTS

Light and Dark:

No significant differences were found in the maximum percentage germination of P. bracteatum seeds imbibed under continuous light or dark at 20ºC. Differences in germination percentage were noted between seed-lots, with germination percent nearly 5% lower both in light and dark for the older seed lot (52 weeks storage).

Table 1: Germination percentage of P. bratceatum seeds 7 and 14 days after imbibition (DAI) at 20ºC in light or dark. Each value is a mean of four replicates (± SEM).

Seed-lot	DAI	Light (%)	Dark (%)
A	7	63.34±1.77	62.67±1.34
	14	76.67±0.67	71.34±1.77
B	7	71.34±2.91	67.34±1.77
	14	84.0±1.16	80.0±1.16
C	7	63.34±1.77	58.67±1.78
	14	80.0±3.53	82.0±1.77

Storage Results:

The proportion of viable seeds in both the graded and ungraded seed lots decreased at relatively constant rates when stored at 20ºC. The graded seed-lot had a significantly higher initial germination percentage (89% ± 2.9) than the ungraded seed-lot (70%± 1.9). The rate of decrease in proportion of viable seeds per week was calculated using the slope of the germination percentage versus time plot, with variability determined at the 95% confidence interval. The rate of decrease in proportion of viable seeds per week was significantly higher for the ungraded seed-lot, 0.22% (± 0.023), than the graded seed-lot, 0.13% (± 0.020).

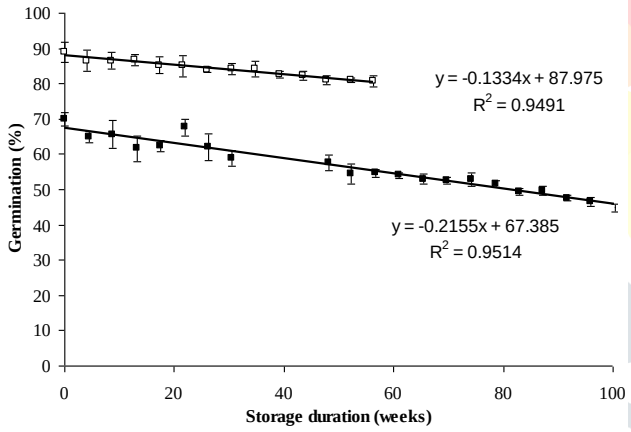


Figure 1: The effect of storage duration (weeks) on the germination of ungraded (■) and graded (□) P. bracteatum seed. Seeds were stored at 20ºC and each point represents the mean of four replicates of fifty seeds ± SEM. The slope and intercept of the regression lines for the two seed lots are significantly different ($p<0.001$).

Effect of Temperature

Logistic regressions fitted to the data provided a good fit for both seed-lots across a wide range of temperatures assessed for one year and two year old seed lots (Figure 2). The maximum germination percentage and time to 50% germination (t-50) were derived from the fitted model. No significant difference was recorded in the maximum germination and rate of germination for temperatures ranging from 15º to 25ºC for both seed-lots (Figure 2). However, there was a significant difference in germination rate and level between this optima range (15-25ºC) and temperatures higher and lower than this for both the one-year ($F_{7, 24}$ =248.16, $P<0.0001$) and two-year old seed-lots ($F_{7, 24}$ =203.511, $P<0.0001$). While both seed lots responded similarly across the optima temperature range, a difference in response was recorded at 30ºC; germination percentage of approximately 85% was recorded in the two year old seed-lot, while it was only 70% in the one year old seed-lot (Figure 2). The one-year seed-lot also exhibited a slightly higher

germination level and lower t-50 at 9°C compared with the two-year old seed-lot suggesting that it was slightly more sensitive to supra-optimal temperature and less sensitive to sub-optimal temperate stress.

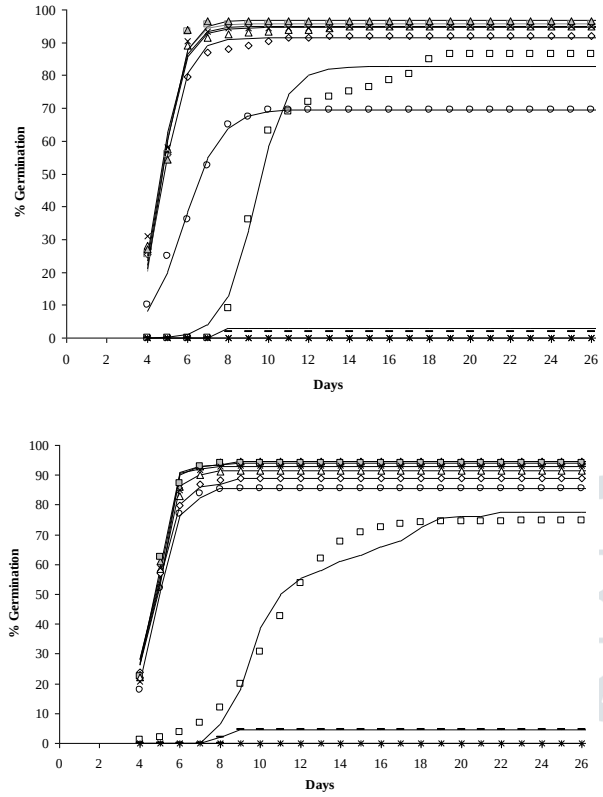
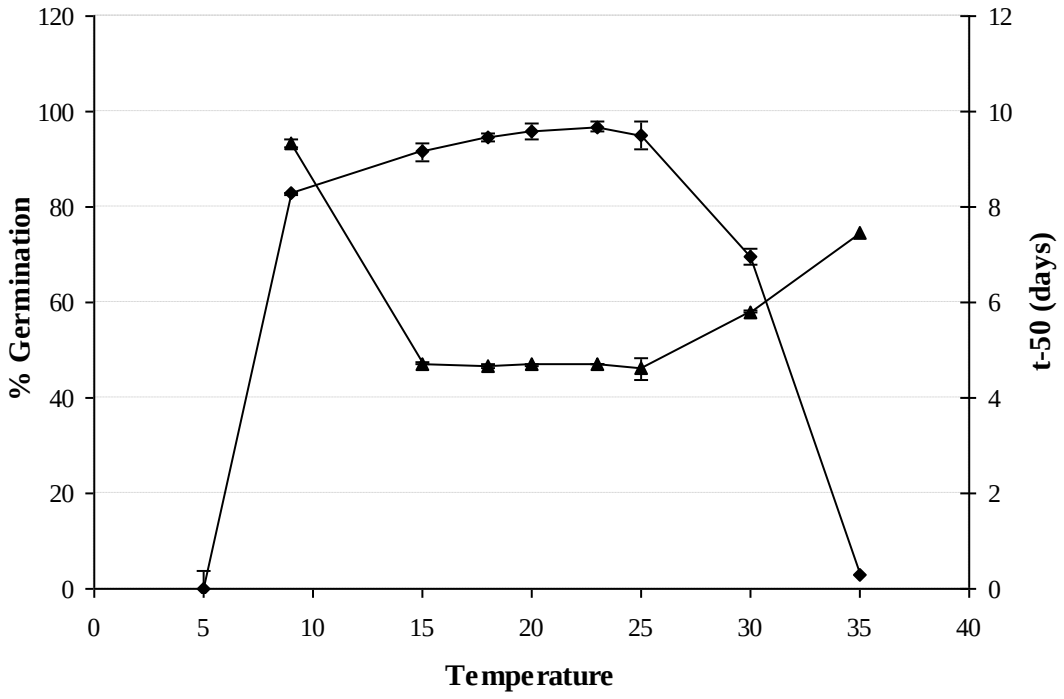
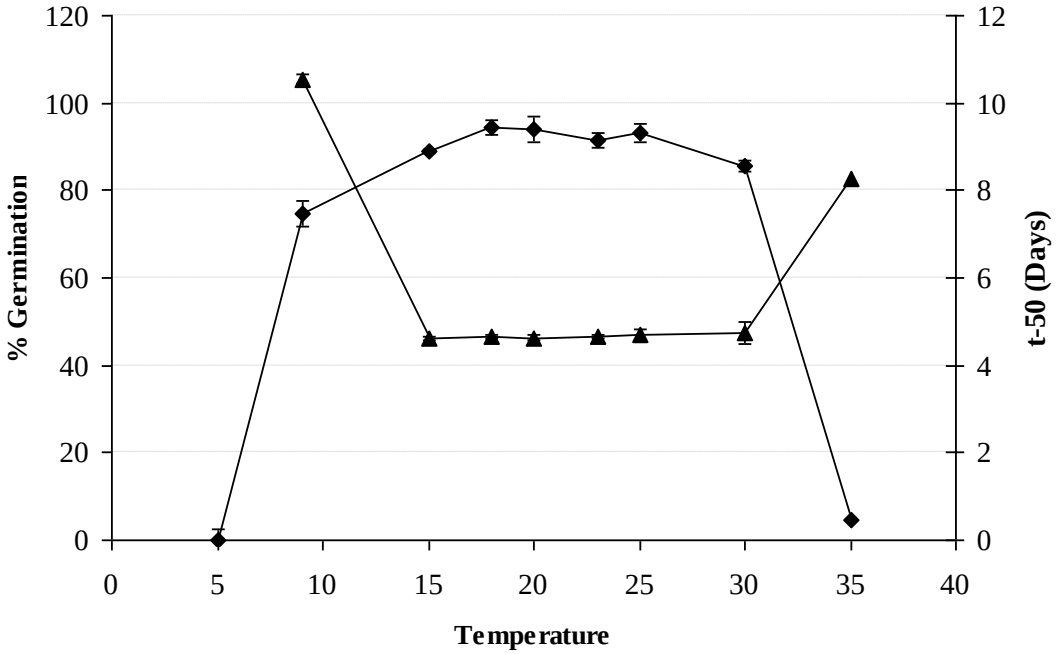


Figure 2: Cumulative germination of (a) one and (b) two year old seed lots of P. bracteatum over a period of 28 days at different temperature regimes. Data points are means of 4 replicates of 50 seeds. Symbols represent (*) = 5ºC; (□) = 9ºC; (◊) = 15ºC; (▲) = 18ºC; (■) = 20ºC; (▼) = 23ºC; (×) = 25C; (○) =30ºC; (▬) = 35ºC. Points are the observed data and solid lines indicate the fitted curves.



(a)



(b)

Figure 3: Maximum of germination (%) (♦) and time taken (days) to fifty percent germination (t-50) (▲) of one (a) and two (b) year old seed lots of Papaver bracteatum germinated at a range of constant temperatures. Bars represent SEM (n=4).

Water Potential:

Germination was completely inhibited at water potentials of -0.1 and -0.3 MPa at temperatures of 20ºC or higher. Only approximately 20% of the seeds were able to germinate at -0.1 and -0.3 at 9ºC and 15% at 15ºC.

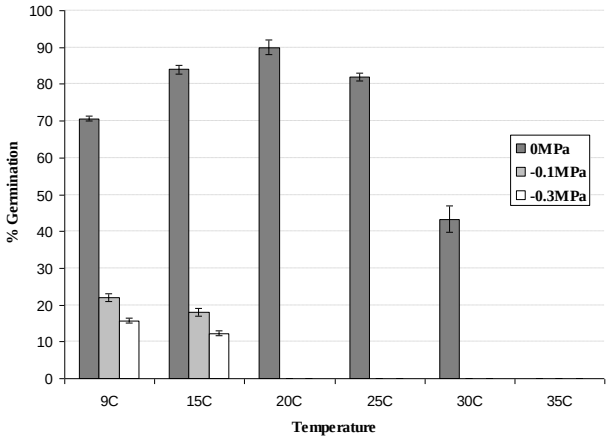


Figure 4: Percentage germination of P. bracteatum seeds at different water potentials (0; -0.1; -0.3 MPa). Each value is a mean of three replicates of fifty seeds. Bars represent SEM (n=4).

DISCUSSION

No physiological dormancy was detected in freshly harvested P. bracteatum seeds, regardless of the growing conditions of the mother plant; similarly, no physiological dormancy was detected in seeds stored for up to 3 years. Although thermal induced secondary dormancy has been reported in P. bracteatum at high temperatures (Bare et al., 1978), this response was not confirmed in the present study. However, the germination response of seeds at supra-optimal temperatures was in general agreement with Bare et al.

(1978) who reported a decrease in germination at 27ºC and no germination at 32ºC. In the present study, a small proportion (<5%) of seeds germinated at 35ºC.

No previous studies have been conducted on the effect of water potential and the results of this present study showed that P. bracteatum seeds were sensitive to water deficits. This sensitivity was exacerbated at high temperature. P. bracteatum originates from temperate, mountainous regions of Iran (Sharghi and Lalezari, 1967) where high temperatures and low rainfall are common in summer and was previously reported to be drought resistant (Neild, 1987). The inability of P. bracteatum seeds to germinate at low water potentials and high temperatures suggests that, while established plants may be able to survive under drought conditions, the capacity to establish crops from seed in areas with insufficient soil moisture will be limited. The slow rate of germination, sensitivity to low water potential and the low proportion of seeds that germinate at low temperatures are considered constraints to industry flexibility in sowing time and site selection in cooler-temperate regions, such as Tasmania. As capacity to germinate at low temperatures is an important attribute in cool temperate regions, seed sowing during warmer months or seed priming should be considered for future seed evaluations.

A loss of seed viability with increasing duration of storage of between 0.1 and 0.3% per week over a 3 year period was recorded in P. bracteatum with a poor quality, ungraded seed lot losing viability at a higher rate than the high quality, graded seed lot. It was concluded that seeds of P. bracteatum could be stored for a period of nine months at 20˚C with a slight decrease in viability, and for up to two years with a reduction in germination percentage of around 20%. These results demonstrate that adequate maintenance of seed quality for commercial plantings may be obtained without specialised storage treatments, with scope for longer term storage but further research would be needed to identify optimum storage conditions. Low temperature and low seed moisture are the two effective means of maintaining seed quality in storage (Bonner, 2003).

The optimal temperature to achieve maximum germination percent was between 18º and 26ºC. The optimal germination temperature range of 15°C to 25°C recorded in the present study is therefore consistent with the previous study. The highest germination percentage and rate of germination, recorded as time taken to reach 50% germination (T50), was 23ºC and germination was inhibited at temperatures of 5ºC and 35ºC. Previously no germination was reported below 10ºC by Bare et al. (1978). The knowledge of the germination characteristics of P. bracteatum seeds will assist the Tasmanian industry to select and manage establishment conditions for the crop.

References

1) DPIWE (2002) Industry Profile of Poppies-Annual Report Tasmania http://www.dpiw.tas.gov.au/inter.nsf/nrmanual2001-02/20/5B1/5D.pdf (accessed August, 2006)
2) Bewley JD, Black M (1994) 'Seed: Physiology of Development and Germination.' (Plenum Press, New York).
3) Benech-Arnold RL, Sanchez RA (1995) Modelling weed seed germination. In 'Seed Development and Germination' pp. 545-566. (Marcel Dekker: New York)
4) Hadas A, Russo D (1974) Water uptake by seeds as affected by water stress, capillary conductivity, and seed-soil water contact. I. Experimental study. *Agronomy Journal* **66**, 643-647.
5) Garwood EA (1979) Water use by six grass species and use of soil water. *Journal of Agricultural Science* **93**, 25-35.
6) Billings WD (1976) Arctic and alpine vegetation: Plant adaptations to cold summer climates. In 'Arctic and Alpine Environments'. (Ed. JD Ives, and Barry, RG) pp. 403-443. (Methuen: London)
7) Bewley JD (1997) Seed germination and dormancy. *The Plant Cell* **9**, 1055-1066.
8) Copeland LO, McDonald MB (1995) 'Seed Science and Technology.' (Chapman and Hall: Melbourne, Australia).
9) Gummerson RJ (1986) The effect of constant temperatures and osmotic potentials on the germination of sugar beet. *Journal of Experimental Botany* **37**, 729-741.
10) Bare CE, Toole VK, Genter WA (1978) Temperature and light effects on germination of *Papaver bracteatum* Lindl, *P. orientale* L, *P. somniferum* L. *Planta Medica* **34**, 135-143.

11) Baskin CC, Milberg P, Andersson L, Baskin JM (2002) Non-deep simple morphophysiological dormancy in seeds of the facultative winter annual *Papaver rhoeas*. *Weed Research* **42**, 194-202.
12) Cirujeda A, Recasens J, Taberner A (2006) Dormancy cycle and viability of buried seeds of *Papaver rhoeas*. *Weed Research* **46**, 327-334.
13) Karlsson LM, Milberg P (2007) Seed dormancy pattern and germination preferences of the South African annual *Papaver aculeatum* L. *South African Journal of Botany* **73**, 422-428.
14) Milberg P, Andersson L (1997) Seasonal variation and light sensitivity in buried seeds of eight annual weed species. *Canadian Journal of Botany* **75**, 1998-2004.
15) Michel BE (1983) Evaluation of the water potentials of solutions of Polyethylene Glycol 8000 both in the absence and presence of other solutes. *Plant Physiology* **72**, 66-70.
16) Michel BE, Kaufmann MR (1973) The osmotic potential of Polyethylene Glycol 6000. *Plant physiology* **51**, 914-916.
17) Dumur D, Pilbeam CJ, Craigon J (1990) Use of the weibull function to calculate cardinal temperatures in faba bean. *Journal of Experimental Botany* **33**, 288-296.
18) Shafii B, Price WJ, Swensen JB, Murray GA (1991) Nonlinear Estimation of Growth Curve Models for Germination Data Analysis. In 'Applied Statistics in Agriculture'. Kansas State University, Manhattan. (Ed. GA Milliken, and Schwenke, J.R) pp. 19-42
19) Thompson CR, Thill DC, Shafii B (1994) Germination characteristics of Sulfonylurea-resistant and susceptible Kochia (*Kochia scoparia*). *Weed Science* **42**, 50-56.

20) Bare CE, Toole VK, Genter WA (1978) Temperature and light effects on germination of *Papaver bracteatum* Lindl, *P. orientale* L, *P. somniferum* L. *Planta Medica* **34**, 135-143.
21) Shafii B, Price WJ, Swensen JB, Murray GA (1991) Nonlinear Estimation of Growth Curve Models for Germination Data Analysis. In 'Applied Statistics in Agriculture'. Kansas State University, Manhattan. (Ed. GA Milliken, and Schwenke, J.R) pp. 19-42
22) Thompson CR, Thill DC, Shafii B (1994) Germination characteristics of Sulfonylurea-resistant and susceptible Kochia (*Kochia scoparia*). *Weed Science* **42**, 50-56.
23) Sharghi N, Lalezari I (1967) *Papaver bracteatum* Lindl*:* A highly rich source of thebaine. *Nature* **213**, 1224-1244.
24) Neild RE (1987) Use of climatic data to identify potential sites in the United States for growing *Papaver bracteatum* as a pharmaceutical crop. *American Metereological Society* **26**, 1117-1123.
25) Bonner FT (2003) Storage of seeds-Potential and limitations for germplasm conservation. *Forestry Ecology and Management* **35**, 35-43.