

Seed Germination and Seedling Emergence of Indian jointvetch (*Aeschynomene indica* L.) in Different Conditions

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자귀풀 종자의 발아 및 출아에 미치는 환경요인

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ABSTRACT Several experiments were conducted in growth chambers and a greenhouse to determine the influence of various environmental factors on seed germination and seedling emergence of Indian jointvetch. Fully matured seeds of Indian jointvetch germinated only 42%. The germination percent increased as the storage temperature increased with time. More than 90% seeds germinated when the seeds were kept at 40°C for seven months, but germination was 58.9 and 55.1% when kept at 25 and 4°C, respectively. Non-dormant seeds of Indian jointvetch germinated 91.1 and 92.4% at 30 and 30/25°C, respectively. Germination percent increased with increasing both prethermal temperature and time. The prethermal temperature of 90°C for 40 minutes was the best for maximum germination (94.5%). Germination and growth of Indian jointvetch tended to decrease slightly until -0.3 MPa osmotic potential (water stress induction) and then declined drastically and the seeds did not germinate at below -0.5 MPa osmotic potential. Indian jointvetch seems to grow well in moist and flooding conditions since emergence and growth of seedling increased with increasing soil moisture content and the water level.

Key words: emergence; environmental conditions; germination; Indian jointvetch; seedling growth.

INTRODUCTION

The seed is an important stage in plant's life cycle for its survival as a species. It is the dispersal unit of plant, which is able to survive between the period of seed maturation and the establishment of

the next generation as a seedling. To optimize germination over time, the seed enters a dormant stage. Dormancy prevents pre-harvest germination as well. Numerous studies have been performed to better understand how germination is controlled by various environmental factors and applied chemicals.

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However, still very little is known about the process by which the embryo emerges from the seed to complete germination and how embryo emergence is blocked in dormant seeds (Bewley 1997). Weed seed germination is influenced by many environmental factors, such as temperature, light, soil salinity, pH, and moisture (Chauhan and Johnson 2008 a,b). A light requirement for seeds to germinate, for example, is a principal means by which germination can be restricted to zones close to the soil surface. Seed burial depth has been shown by others to affect seedling emergence of several weed species (Benvenuti *et al.* 2001; Boyd and Van Acker 2003). Information on the requirements of a species for germination and emergence can be used to characterize the competitiveness and the potential infestation range of the weed, and could contribute to the development of effective weed control options.

Indian jointvetch is an important species in both crop and noncrop areas. This weed has the potential to become a serious problem in and around rice fields for several reasons. Infestations may interfere with harvesting, reduce yields, and decrease profits due to extensive seed cleaning costs (Indian jointvetch seeds are very difficult to separate from rice during the milling process). This weed has a prolific seed production with the seeds dispersing within four weeks of flowering (Charles 1999). It germinates in spring and summer and will grow rapidly over summer, flowering in summer and autumn. Most frequently found in poorly drained areas, wet pastures, along rivers and canals, and in or around paddy fields (Holm *et al.* 1997). In Guam, Indian jointvetch was found in man-disturbed areas and thickets, not common (Stone 1970). In Fiji, this weed occurred as a weed from near sea level to low elevations on gravel banks of rivers, along roadsides, in waste places, and often in wet parts of rice fields and cane fields (Smith 1985). In New Guinea,

Indian jointvetch is known a plant of wet situations; stream banks, swamp margins, roadside ditches, and sometimes as a weed in pastures. Indian jointvetch is widely distributed from low altitudes to 800 m (Henty and Pritchard 1975).

Information of Indian jointvetch is very limited and a few germination and control measure data are available. Effects of seed maturity, storage condition, thermal pretreatment, light, temperature, and moisture stress on germination and seedling growth were studied as part of eco-physiology study of Indian jointvetch to get basic information for control of this weed species.

MATERIALS AND METHODS

Seed collection

Pods of Indian jointvetch were collected at maturity (when the plant had senesced) from Chungnam National University Experimental Farm, Daejeon, Korea. Pods from more than 100 plants were combined to obtain experimental samples. Pods were threshed and the seeds were separated from the chaff manually, placed in polythene bags, and stored at room temperature (25°C) until using in the experiments.

Germination tests

Seed germination was determined by placing 25 seeds in 100×40 mm dimension sterile Petri dishes over the surface of sterile Whatman #1 filter paper (90 mm diameter) and 10 ml of distilled water. Then all the Petri dishes were put in a growth chamber at the specified temperature under standard cool white fluorescent tubes with a flux rate of 35 $\mu\text{mol s}^{-1} \text{m}^{-2}$ and a 14-h photoperiod. For germination in complete darkness, the Petri dishes were wrapped in a double layer of aluminum foil. The number of

the germinated seeds was counted two weeks after starting each experiment.

Grading of seeds based on color

After harvesting and threshing, the seeds were put together without grading. Seeds were graded based on their color. There were three different colors of seeds such as black (full mature), dark green (medium mature) and light green (immature seeds) in the collected samples.

Seed storage in different temperature and duration

After grading the seeds, the full matured (black color) seeds were selected and around 300 seeds were kept in each polyethylene bag and then seeds were put in three different temperatures i.e., 4, 25 and 40°C for several months. In every one month interval seeds kept in three different temperatures were brought for placing in germination test until 7 months. Four replicates were used for each time.

Effect of temperature

To identify a suitable temperature for subsequent experiments, germination was determined in growth chambers at different constant temperature (10, 15, 20, 25 and 30°C) as well as alternative day/night temperatures (15/10, 20/15, 25/20, 30/25°C) at 14 hr/10 hr under standard cool white fluorescent tubes with a flux rate of 35 $\mu\text{mol s}^{-1} \text{m}^{-2}$ at 14 hr photo-period. The seeds which stored for seven months at 40°C were used for this experiment.

Thermal pretreatment of seeds

To determine the effect of high temperatures on germination, seeds were placed in the ovens maintaining different temperatures i.e., 60, 70, 80 and 90°C for durations of 10, 20, 30, 40 and 50 minutes. The treated seeds were then put in a growth chamber at 30/25°C (day/night temperature) at 14

hr/10 hr for two weeks to determine the germination percentage. A control treatment in which seed were stored at room temperature was also included.

Germination under light and dark condition

Germination of both dormant and non-dormant seeds were determined in growth chambers under day/night temperature (30/25°C) at 14 hr/10 hr in both light/dark and dark regimes.

Germination under water stress

To assess the effects of water stress, aqueous solutions with osmotic potentials of 0, -0.1, -0.2, -0.3, -0.4, -0.5 and -0.6 MPa were prepared using specific concentrations of PEG 6000 in distilled water. The following equation (Agrawal and Dadlani 1992; Burlyn and Kaufmann 1973) was used to calculate osmotic potential from known concentrations of PEG 6000 in 25°C :

Osmotic potential =

$$-(1.18 \cdot 10^{-2})C - (1.18 \cdot 10^{-4})C^2 + (2.67 \cdot 10^{-4})18CT + (8.39 \cdot 10^{-7})C^2T,$$

Where C is the concentration of PEG (kg^{-1} distilled water) and T is the temperature (in degrees C). One preliminary germination test was made. Eight osmotic potentials were used in the first experiment (0, -0.2, -0.4, -0.6, -0.8, -1.0, -1.2 and -1.4 MPa). In the second experiment, seeds were exposed to osmotic potentials of 0, -0.1, -0.2, -0.3, -0.4, -0.5, -0.6, MPa. Four replicates sets were used for germination.

Soil moisture effect

Field capacity of water in soil

To see the growth pattern of Indian jointvetch different moisture situation was created and then seeds were grown on glass box (40 cm length \times 45 cm height \times 9 cm width). Three different moisture

conditions were maintained i.e., 30-40%, 90-100% of field capacity and flooding water condition (2~3 cm water).

Water depth

The effect of different water levels on seedling growth of Indian jointvetch was studied in glass box. Fifty non-dormant seeds of Indian jointvetch were placed on the soil surface in (40 cm length × 45 cm height × 9 cm width) transparent plastic glass box and then covered with soil. Then water was added to achieve water levels of 3, 6, 9, 12, 15, 18, 0, -3 and -6 cm. The positive values indicate water level remained above the place of seed placement and the negative values indicate below the seed placement level. The value zero (0 cm) means water level was in the same position of seed placement. Soil used for this experiment was passed through a 3 mm sieve before conducting the experiment.

Statistical Analysis

In the laboratory experiments, each replication was arranged on a different shelf in the germination chamber. Treatments of each experiment were replicated four times and all experiments were repeated. All data were analyzed with the SAS Software release 9.1 (SAS Institute Inc. 2006, Cary, NC, USA). Analysis of variance was performed and means were tested with Duncan's multiple range test. Standard deviations are also provided to show the variations associated with particular means.

RESULTS AND DISCUSSION

Effect of seed color

Seed color is one of the most important characteristics of seed maturity. We found three different

Table 1. Effect of seed maturity on germination of Indian jointvetch at 25°C based on the color of seeds.

Seed color	Germination (%)
Black	42.3 a ^a
Dark green	34.4 b
Light green	0 c

^aMean values in a column having the same letter do not differ significantly at 5% level by DMRT.

colors of seeds at maximum maturity stages. The germination was 42.3% (Table 1) when the seeds attained at fully mature stage (Black color appeared). The second categories seeds (dark green) were able to germinate 34.4%, but no germination was observed when the seed was immature (light green color). Though fully mature seeds germinated better, it showed too low germination (only 40%) which means Indian jointvetch seeds remained in a dormant stage in a normal condition. Cuthbertson (1970) found that germination percentage of *Chondrilla juncea* varied with the color of the seeds. As the developing seeds of *Chondrilla juncea* matured the color changed from greenish-white through yellow to yellow-brown or olive-green. Some 25% of the yellow seeds were capable of germination, whereas fully colored and mature seeds showed 80 to 90% germination and remained viable for two to three years when stored in open containers.

Seed storage in different temperature and duration

From our previous results it was seen that only 40% of the seeds could germinate when stored at room temperature. Seeds were stored in different temperature for several months for breaking seed dormancy of Indian jointvetch. The germination percentage increased as increasing the storage temperature with time. More than 90% seeds germinated when the seeds were kept at 40°C for seven months (Fig. 1). The differences were not so

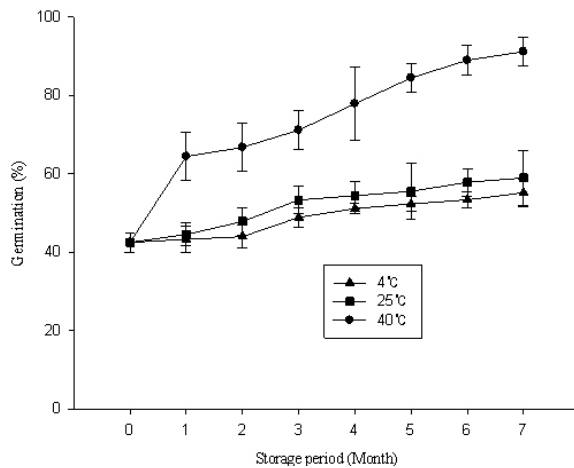


Fig. 1. Effect of different storage conditions on germination of Indian jointvetch.

much from five months to seven months. The germination percentage was almost the same when the seeds were placed at 4 and 25°C for several months. The highest germination was 55.1 and 58.9%, when the seeds were kept in 4 and 25°C, respectively for seven months. But the germination percentage was significantly varied with the storage temperature. Our results are in agreement with other studies that germination percentage increased with storage for several days in switchgrass (Mark *et al.* 1994) in wild sunflower (Seiler 1998) and partially agreed to the results of Kyoung *et al.* (2002) who reported that Indian jointvetch seeds showed higher germination percent at 35°C for 4 months storage, but we found the highest germination percentage at 40°C after 7 months storage.

Effect of temperature

Seed (non-dormant) germination was influenced with temperature (Table 2). The highest germination was observed at 30°C. The germination percentage of seeds was very low at 10 and 15°C and 54.5% seeds germinated at temperature 20°C. The data indicate that non-dormant seeds of the Indian jointvetch germinate at 25°C~30°C. Although there

Table 2. Effect of temperatures on germination of Indian jointvetch.

Temperature (°C)	Germination (%)
10	8.9 d ^a
15	22.3 c
20	54.5 b
25	88.9 a
30	91.1 a

^aMean values in a column having the same letter do not differ significantly at 5% level by DMRT.

Table 3. Effect of alternative temperatures on germination of Indian jointvetch.

Temperature (°C)	Germination (%)
15/10	16.7 c ^a
20/15	55.5 b
25/20	90.0 a
30/25	92.4 a
35/30	83.5 a

^aMean values in a column having the same letter do not differ significantly at 5% level by DMRT.

was not so much difference between constant and alternative temperature for germination of Indian jointvetch, alternative temperature showed slightly better germination than constant temperature (Table 3). The alternative temperature 30/25°C was the optimum condition for giving higher germination, but germination percentage decreased at above or below 30/25°C.

Germination under light and dark condition

Germination of both dormant and non-dormant seeds of Indian jointvetch was not much influenced in light and dark conditions (Table 4). When the dormant seeds were put for germination in light condition, the germination was 5% higher than in dark condition. For non-dormant seeds germination percentage did not vary significantly though dark

Table 4. Effect of light on germination and shoot growth of dormant and non-dormant Indian jointvetch at 25°C.

Seeds	Light condition	Germination (%)	Shoot length (cm)
Dormant seed	Light	45.0 b	6.2 c ^a
	Dark	40.0 b	10.1 a
	Light+Dark	43.7 b	8.7 b
Non-dormant seed	Light	90.0 a	8.1 b
	Dark	93.8 a	10.9 a
	Light + Dark	92.5 a	10.5 a

^aMean values in a column having the same letter do not differ significantly at 5% level by DMRT.

condition was slightly higher than others. The finding that germination of Indian jointvetch was not affected by light is in agreement to other studies that show that light indifference in many species in the Fabaceae family is widespread (Baskin *et al.* 1998; Silveria and Fernandes 2006).

Effect of thermal pretreatment

Thermal pretreatment with time was effective for higher germination of Indian jointvetch seeds (Table 5). It was shown that germination percentage increased with increasing both prethermal temperature and time. Only 34% of the seeds germinated when stored at room temperature. The germination percentage was almost double when Indian jointvetch seeds

were treated with 60°C for 10 minutes. Germination percentage also increased by further increasing the duration of prethermal treatment in the same temperature. The prethermal temperature of 90°C for 40 minutes was the best for maximum germination. The germination percentage was similar at 80 and 90°C prethermal treatments irrespective of duration. This result is in agreement to the results of Kyoung *et al.* 2002 who found that thermal pretreatment was effective in germination enhancement in Indian jointvetch. Thermal treatment was effective for the germination of *Grevillea* species (Edwards and Whelan 1995; Morris 2000) and also for some leguminous crop (Shea *et al.* 1979; Auld and O'Connell 1991) which fully agreed to our findings.

Effect of water stress

In the first experiment, germination was not observed at -0.6, -0.8, -1.0, -1.2 and -1.4 MPa osmotic potential conditions, but seed germination at 0, -0.2, and -0.4 MPa osmotic potential was 87.0, 54.0 and 21.0%, respectively (results not shown). In the second experiment germination of *Aeschynomene indica* at 25°C was tested at 0, -1, -2, -3, -4, -5 and -6 MPa, and germination was 91.5, 88.8, 84.3, 78.0, 55.5, 22.8 and 0%, respectively (Fig. 2). Our results confirmed the results of Singh and Singh (1982),

Table 5. Effect of thermal pretreatment on germination of Indian jointvetch at 25°C.

Temperature (°C)	Time (minutes)				
	10	20	30	40	50
	%				
60	67.5 b	73.1 c	74.4 c	74.8 d	77.3 c ^a
70	68.9 b	74.5 c	81.4 b	81.9 c	82.2 b
80	88.3 a	82.6 b	82.1 b	88.1 b	83.3 b
90	92.3 a	91.1 a	93.1 a	94.5 a	93.1 a
Control	34.4 c	34.4 d	34.4 d	34.4 e	34.4 d

^aMean values in a column having the same letter do not differ significantly at 5% level by DMRT.

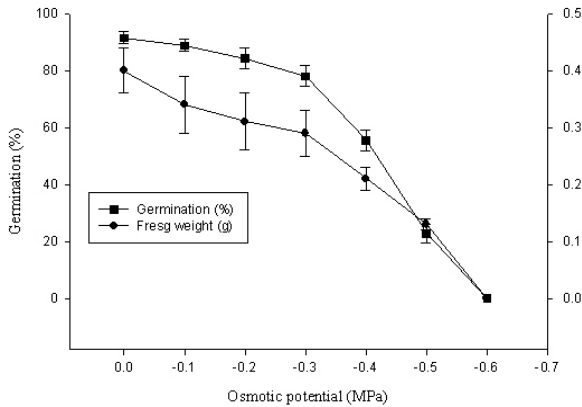


Fig. 2. Effect of osmotic potential on seed germination and shoot weight of Indian jointvetch.

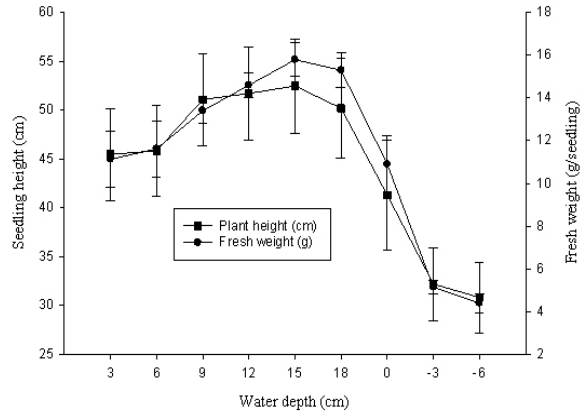


Fig. 3. Effect of water depth on seedling height and fresh weight.

who found that the germination percentage generally decreased with decreasing water potential, and also results of Ghorbani *et al.* (1999), who reported that germination of *Amaranthus retroflexus* increased with increasing osmotic potential. The trend of growth of plants (fresh weight basis) was similar to germination as increasing osmotic potential the fresh weight was increased.

Effect of soil moisture availability

Increasing the moisture availability to the seeds of Indian jointvetch, growth of seedling was increased (Table 6). It was observed that Indian jointvetch plants grew rapidly (considering plant height and fresh weight) under high field capacity moisture condition (Table 6).

A wide range of variation was found for the growth of Indian jointvetch at different water depth conditions. The increasing trend of seedling growth remained up to 15 cm water depth, and then started to decline (Fig. 3). In terms of fresh weight, it was seen that 73% higher fresh weight was observed when the water level remained at 15 cm compared to -6 cm from the seed placement. Indian jointvetch prefers a moist condition for growth, so rice field is a favorable place for growing Indian jointvetch. From the previous report it was seen that Indian

Table 6. Effect of soil moisture on growth of Indian jointvetch seedling.

Soil moisture content (Field capacity, %)	Plant height (cm)	Fresh weight (g/seedling)
30-40	17.4 c	1.5 c ^a
90-100	23.3 b	5.5 b
Flooding water (2-3 cm)	28.9 a	10.4 a

^aMean values in a column having the same letter do not differ significantly at 5% level by DMRT.

jointvetch most frequently found in poorly drained areas, wet pastures, along rivers and canals, and in or around paddy fields (Holm *et al.* 1997) in waste places, and often in wet parts of rice fields and cane fields (Smith 1985), widely distributed from low altitudes to 800 m (Henty and Pritchard, 1975). From our study we also found the same results that Indian jointvetch grew better in a moist soil.

요 약

여러 환경요인이 자귀풀의 발아 및 출아에 미치는 영향을 성장상과 온실에서 조사하였다. 성숙된 자귀풀 종자의 발아율은 42%로 낮았으나 저장기간이 길어짐에 따라 발아율이 증가하였다. 40°C 온도조건에서 7개월간 종자를 보관하였을 때는 90% 이상 발아

율이 증가되었으나 25°C와 4°C에서는 각각 58.9%와 55.1%로 낮게 발아되었다. 휴면 각성된 자귀풀 종자는 30°C 항온에서는 91.1% 발아되었고, 30/25°C 변온에서는 92.4% 발아되었다. 90°C 고온에서 40분간 전처리한 경우에는 발아율이 94.5%로 가장 높았다. 삼투포텐셜 조절에 의하여 수분스트레스를 유기하였을 때 -0.3 MPa까지는 자귀풀의 발아와 초기 생장은 완만하게 감소되었으나 그 이하 수준으로 낮아질수록 크게 감소되어 -0.6 MPa에서는 전혀 발아되지 않았다. 자귀풀은 포장용수량이 높거나 담수상태 조건에서, 그리고 수심이 깊을수록 초기 성장량이 높은 것으로 미루어 습윤한 밭 조건이나 담수상태의 논에서 잘 생육하는 것으로 사료된다.

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