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Germination and Seedling Development of *Trapa bispinosa* Roxb

[1]O. Ima Mirani, [2]Z. Muta Harah and [1]B. Japar Sidik
[1]Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus, Nyabau Road, 97008 Bintulu, Sarawak, Malaysia
[2]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Abstract: *Trapa bispinosa* is a freshwater macrophyte occurring in stagnant or slow moving water streams. Information on assessment of seeds storage and germination of *Trapa bispinosa* is less available in literature. Dependence on fresh seeds abundance only from natural environment for plant propagation or cultivation may lead to insufficient supply of seeds due to various biotic and abiotic factors. This study evaluated the viability and germination of *Trapa bispinosa* seeds stored in zip-lock plastic bag at low temperature of 7°C for six months and fresh seeds. In addition germinating seeds progressive development to juvenile plants was recorded and described. Experiments were conducted where stored and fresh seeds were soaked in 62×45×54 cm glass tanks filled with aged tap water to the level of 15 cm depth. Stored seeds showed low percentage germination of 2.82% compared to fresh seeds which was 71.19%. Eight distinct developmental stages were identified from germinating seeds to juvenile plants. Both stored and fresh seeds produced plants of similar morphology but stored seeds progressive development from germination, seedling to juvenile plants needed longer duration to achieve. The storage of seed at low temperature at 7°C for six months showed reduced viability and also vigorousity. Improved methods should be developed for *Trapa* seeds storage taking into account of the seeds' endurance to dryness and moisture levels in order to maintained seeds viability for future uses either for production, research purposes or even conservation and restoration programs.

Key words: Seed storage, juvenile plant development, germination duration, *Trapa bispinosa*

INTRODUCTION

Trapa is a freshwater macrophyte occurring in stagnant or slow moving water. This annual plant bears single seeded edible fruits (Ghosh, 2004) which are viable for propagation (Rubaztky and Yamaguchi, 1977). Being annual, e.g., *Trapa natans* L. overwinter entirely by seeds, where detached seeds sank to the bottom and exhibited a period of dormancy (Methe *et al*., 1993). However, viability of buried seeds within the natural environment varies and can be viable in excess of five years (Kunii, 1988a). Only a portion of seeds produced each year germinate the following spring while those remaining, accumulate to produce a seed bank in the sediment (Methe *et al*., 1993). Other studies that have been carried out to include seasonal growth and biomass of *T. japonica* Flerov in natural condition (Kunii, 1988b), micropropagation of three different *Trapa* varieties; *T. quadrispinosa* Roxb, *T. bispinosa* Roxb and *T. bicornis* Nakano at Rajshahi Division, Bangladesh (Hoque *et al*., 2006), germination and survival of *T. japonica* Flerov plants to produce seeds (Kunii, 1988b) and, germination and survival of *Trapa natans* var. *japonica* Nakai (Kurihara and Ikusima, 1991). Information on the mechanism of preservation of *Trapa* seed was less available in literature. Muenscher (1936) reported that *T. natans* seeds loss viability by dryness and were released from dormancy by chilling but the period of storage was not discussed. This present study examined the germination success and development of germinating seed to juvenile plant of *T. bispinosa* for both stored and fresh seeds.

MATERIALS AND METHODS

Plant material: Fresh *Trapa bispinosa* seeds imported from China obtained from local markets were used as plant materials for this study. Fresh seeds was placed in zip lock plastic bag and kept at low temperature at 7°C in a refrigerator for six months. Another batch of fresh seeds was acquired 6 months later and used as a comparison to the seeds stored in the zip lock for

Corresponding Author: Z. Muta Harah, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia Tel: +603-89474890

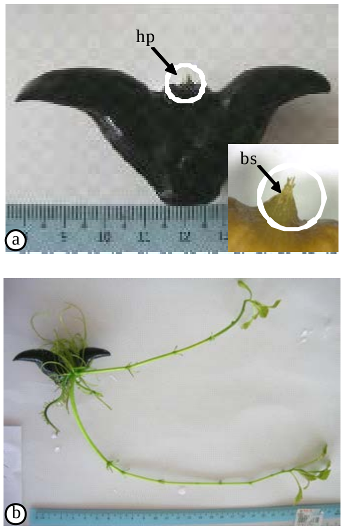

Fig. 1(a-b): (a) The appearance of a white hypocotyl (hp) with barbellate structure (bs) at the seed apex and (b) A juvenile plant of *T. bispinosa*

germination experiment and observation of growth stages from germinating seeds development to juvenile plants.

Seed germination and development of germinating seed to juvenile plant: One hundred seventy seven stored seeds in zip lock plastic bag kept at low temperature at 7°C in a refrigerator for six months and 177 fresh seeds were measured for horn to horn length, height and fresh weight using vernier caliper and analytical balance to two decimal places, respectively. Seeds of both batches were germinated separately in a 62×45×54 cm glass tanks filled with aged tap water to the level of 15 cm depth and kept at temperature of 25-26°C and exposed under normal day light. Germination was detected by the appearance of the seed's hypocotyl (Fig. 1a). Number of seeds germinated and the progressive development of germinating seeds were observed and recorded up to two months. As the size of the seedlings increased, they were transferred into a bigger glass tank for further observation until the stage of juvenile plant with well developed floating leaves possessing swollen petiole with the seed still attached to the plant (Fig. 1b).

RESULTS

Seed germination: Germination of *T. bispinosa* seeds were detected by the emergence of a white hypocotyl from a germination pit or hole at the seed apex with barbellate structure (Fig. 1a). In the period of 60 days, percentage germination for stored seeds was 2.82%

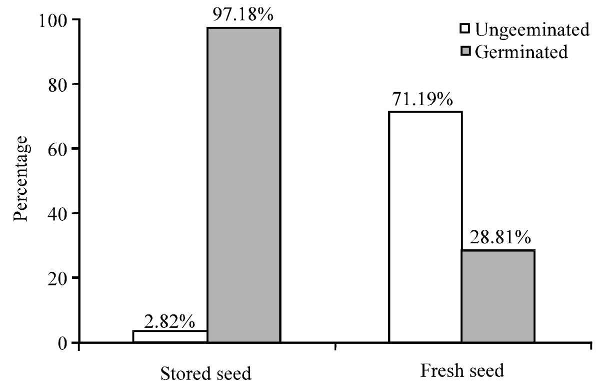

Fig. 2: Germination of stored and fresh seeds of *T. bispinosa*

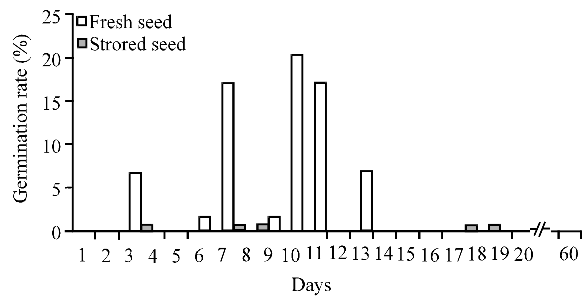

Fig. 3: Seeds germination of stored and fresh seeds

(5 seeds) while fresh seeds showed 71.19% (126 seeds) (Fig. 2). Non-germinated seeds showed percentage of 97.18% (172 seeds) and 28.81% (51 seeds), respectively for storing and fresh seeds. Maximum percentage germination in stored seed was achieved within a period of 19 days compared to fresh seeds that occurred within a period of 13 days. Fresh seed showed more germination than stored seeds during the duration of two months soaking in water. For stored seeds only one seed was able to germinate each day, in contrast to fresh seeds where the highest number for seed germination occurrence was 36 seeds on day 10 (Fig. 3). Prior to planting, seed dimensions were determined based on the physical characteristics of horn to horn length, height and weight. Seed dimensions and weight of both categories have no effect on germination (Table 1).

Development of germinating seed to juvenile plant: Irrespective of different starting plant materials, stored versus fresh seeds, eight distinct progressive developmental stages of *T. bispinosa* were identified and are described as; Stage 1: appearance of a white hypocotyl from seed apex. Hypocotyl later turned green in color (Fig. 4a), Stage 2: hypocotyl curved upward

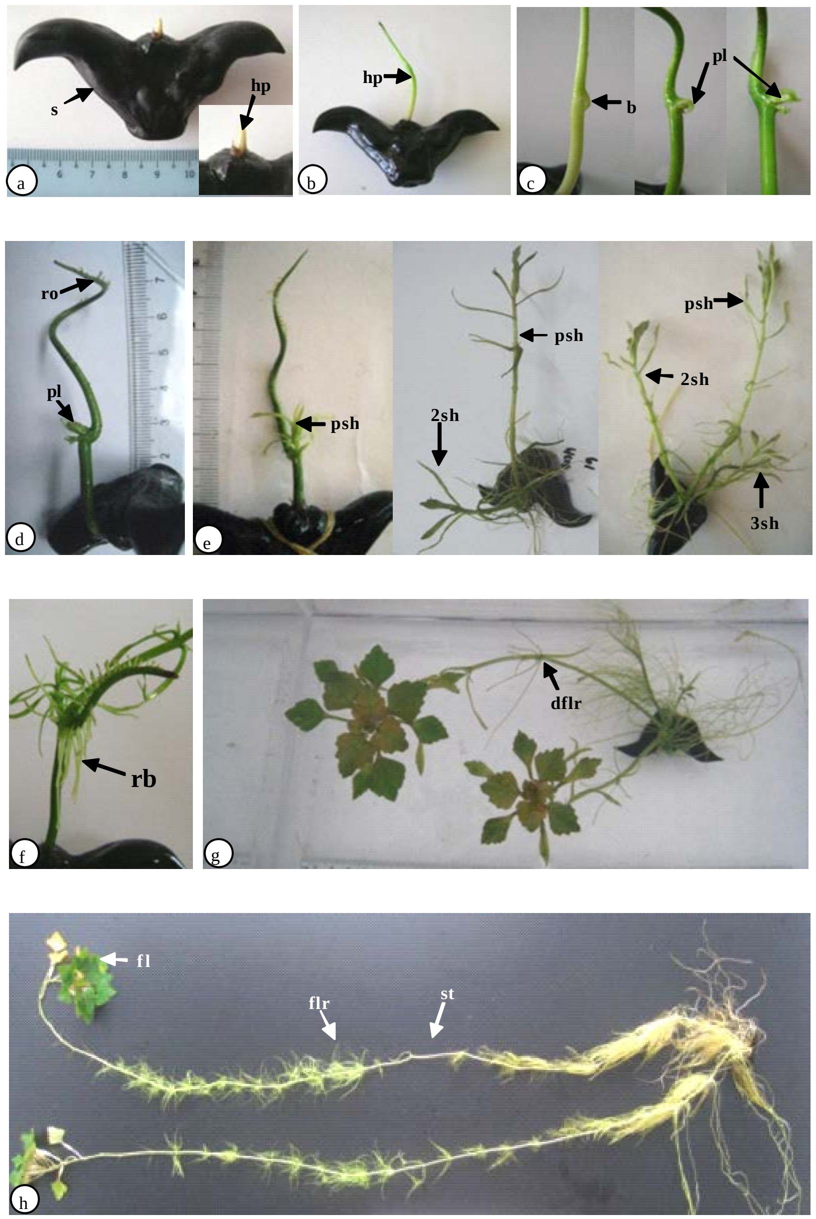

Fig. 4(a-h): Development of germinating seed (from fresh seed) to juvenile plant (a) Stage 1: Day 3, appearance of a white hypocotyl from seed apex, (b) Stage 2: Day 4, hypocotyl curved upward and spiral in shape and became elongated, (c) Stage 3: Day 7-8, emergence of plumule from the protrusion on the hypocotyl, (d) Stage 4: Day 8-9, emergence and growth of branched roots from hypocotyl in a row starting from its upper part, (e) Stage 5: Day 11-17, formation of pioneer shoot, followed by a succession of second and third shoots, (f) Stage 6: Day 12-21, roots at the base of hypocotyl grew downward showing positive geotropism in undeterminate direction, (g) Stage 7: Day 12-25, this stage was characterised by a shoot or shoots producing leaves, (h) Stage 8: Day 14-27, juvenile plant, morphologically resembles the adult plant, except the seed is still attached to the plant, s: Seed, hp: Hypocotyl, b: Protrusion, pl: Plumule, ro: Roots on hypocotyls, psh: Pioneer shoot, 2sh: Second shoot, 3sh: Third shoot, rb: Roots below hypocotyl, dflr: Developing featherlike root (resembles fine thread), flr: Featherlike root, fl: Floating leaves, st: Stem

Table 1: Comparison of seed dimensions and weight of stored and fresh seeds. The values represent the means±SD. Means with the same alphabet in the same row is not significantly different (T-test, $p<0.05$), SD-Standard deviation

	Category of seed	
Seed dimensions parameters	Stored seed	Fresh seed
Length of horn to horn (mm)	70.84±5.24[a]	62.85±8.78[a]
Height (mm)	27.10±2.14[a]	24.42±2.09[a]
Wet weight (g)	12.52±3.40[a]	8.28±3.32[a]

and spiral in shape and becomes elongated (Fig. 4b), Stage 3: emergence of plumule from protrusion on the hypocotyl (Fig. 4c), Stage 4: emergence and growth of branched roots from hypocotyl in a row starting from its upper part (Fig. 4d), Stage 5: formation of pioneer shoot, followed by a succession of second and third shoots (Fig. 4e), Stage 6: roots below the hypocotyl appeared and simultaneously elongated. Roots below the hypocotyl grew downward showing positive geotropism. They also grew in undeterminate direction and clump to each other from the growing stem (Fig. 4f), Stage 7: this stage was characterized by a shoot or shoots producing leaves (Fig. 4g) and, Stage 8: juvenile plant stage, distinguished by the presence of well developed floating leaves with swollen petiole and featherlike roots. These featherlike roots first appear as curved fine thread and later branching resembling feathers. The featherlike roots were light green in colour, oblong in shape and originated from the leaf scars or nodes of the stem. Well developed featherlike roots usually formed two pairs of roots in opposite directions around the stems (Fig. 4h).

Durations for the eight developmental stages varied from 8-17 days, where Stage 5 has the longest period of 17 days. Intervals between developmental stages were 1-7 days. In this study, leafy shoots were first observed emerging and floating on water's surface on day 19 and 14 for stored and fresh seeds, respectively.

DISCUSSION

Germinating seeds were only detected after a brief period of 3 days after immersion in water. Each seed grows with the appearance of a white hypocotyl emerging from the seed apex. In natural environment, seeds may have been superficially buried and remain dormant until favorable condition allows the seed to germinate. In this study seeds immersed in water, exposed to lighted condition induced them to germinate. Stored seeds have very low percentage germination compared to fresh seeds with percentage germination approximately reduced by 25 folds. Only 5 seeds of 177 stored seeds were able to germinate in contrast to 126 of 177 fresh seeds. *Trapa* seed cotyledon contains 80% water (Rubaztky and Yamaguchi, 1977) and deteriorates due to moisture loss. Zip lock plastic bags were used for seed storage to reduced moisture loss when compared to direct exposure in low temperature environment. However, moisture loss up to 4.63% has been detected in okra, *Abelmoschus esculentus* (even though its seeds contained 85% water) stored in a plastic bag at 10°C (Adetuyi *et al*., 2008). Stored seed in particular when stored in cooler drier low temperature places such as refrigerator, lost its moisture due to low relative humidity and, under prolonged condition caused dryness to the seed. Loss of moisture resulting in dryness during storage of seeds as demonstrated in *T. natans* consequently decreased the germination rate (Muenscher, 1936). Aquatic plants' negative buoyant seeds such as *Trapa* develop and sink underwater and seldom exposed to dryness and therefore sensitive to dryness even after a few hours of exposure (Kurihara and Ikusima, 1991). Muenscher (1936) believed that storage in water is vital particularly for seeds intended for planting or propagation. This was ascertained by Rubaztky and Yamaguchi (1977) where *T. natans* and *T. bicornis* seeds must be kept moist in order to remain viable. Seeds stored in water-filled glass bottle (9 cm in diameter, 20 cm in depth) under room condition were viable up to 5 years (Kunii, 1988b). In India, harvested fruits of *Trapa* were stored in an earthen pots filled with water with seed fully imbibed in water to maintain the viability for the subsequent planting (Agrawal and Ram, 1995).

Seeds' dimension and weight as given in Table 1 were not significantly different as variations in both parameters were high as indicated by the standard deviation. Findings of Suriyagoda *et al*. (2006) and Arima *et al*. (1999) showed that bigger size seed contributed to *Trapa* spp. survival in natural environment. Large seed has large cotyledon that provides sufficient food reserves where plants can absorb and synthesize food for initial growth. In this present study, heavier stored seeds with large dimension were observed to be unable to germinate. The storage method and prolonged time rather than seeds' size caused seeds to gradually loss their viability. However, seeds if properly dried and cool-stored could retain a high viability over several years thus retaining its reserves (Grubben, 1978). Improved methods should be developed for *Trapa* seeds storage taking into account of the seeds endurance to dryness and moisture levels in order to maintained seeds viability for future uses either for production, research purposes or even conservation and restoration programs.

Both stored and fresh seeds produced plants of similar morphology but stored seeds progressive development from germination, seedling to juvenile plants needed longer period to achieve. This suggests seed storage at low temperature and for prolonged period caused gradual loss of viability and also the vigorousity. The seed germination to progressive development to seedlings and juvenile plants are categorically identified into 8 stages. Stage 1 is the germinating seed, Stage 2-7 are stages of development and growth of seedlings. Seedlings at Stage 7 continued to grow a succession of rosettes or stems with roots and shoots producing floating leaves that emerge to the water's surface. At this stage, the plant is referred to as juvenile plant that closely resembles the adult plant that can reproduce through sexual reproduction. The unique ability to propagate clonally by producing ramets or secondary rosettes (Groth *et al*., 1996), i.e., in this study 2-3 rosettes or stems complete with roots and shoot producing leaves from a single seed enable the species to survive and expand the plant population. In each rosette, the developed roots grow downward with positive geotropism and function to anchor the plant to the soil (Rubaztky and Yamaguchi, 1977). This characteristic is an adaptive feature enabling the plant to colonize areas with slow moving water as well.

CONCLUSION

Stored seeds showed low percentage germination compared to fresh seeds. Stored seeds, kept at low temperature of 7°C for prolonged period, i.e., six months showed reduced viability and also vigorousity. Both stored and fresh seeds produced plants of similar morphology but stored seeds progressive development from germination, seedling to juvenile plants needed longer duration to achieve. Eight distinct developmental stages were identified from germinating seeds to juvenile plants; (1) appearance of a white hypocotyls on seed apex, (2) elongation of hypocotyl, (3) emergence of plumule, (4) formation of roots on hypocotyl, (5) formation of shoots, (6) elongation of roots below hypocotyl, (7) formation of plantlets and (8) juvenile plant. Adopted storage method of seeds storage in earthen pots filled with water as in India or an alternative improved methods should be developed for *Trapa* seeds storage taking into account of the seeds' endurance to dryness and moisture levels in order to maintained seeds viability for plantings, future uses either for production, research purposes or even conservation and restoration programs.

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