Plant Tissue Cult. **14**(2) : 161-166, 2004 (December)

PTC

Various Color Illumination Effect on *In vitro* Multiple Shoot Induction in Water Chestnut (*Trapa japonica*)

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Key words : Color illumination, Multiple shoot, Induction, Water chestnut

Abstract

Shoot production efficiency through cotyledonary nodes as well as nodal explants were studied under various color illumination conditions in water chestnut *(Trapa japonica* Flerov). Under various color (white, blue, mixed, red and intra-red) illuminations, mixed color illumination showed the early and higher percentage of shoot proliferation. Fresh and dry weight were also significantly higher under the mixed color condition. Media consisted of MSMA with 2.7 µM BA, 0.5 µM NAA and 0.5 µM GA_3 in all color illuminations. The competence of cotyledonary node as well as nodal explants for the induction of multiple shoots under the influence of various color illumination as well as plant growth regulators have been observed. Shoots produced *in vitro* rooted 100% in liquid MS supplemented with 5.4 µM IBA. Plantlets were established successfully in soil.

Introduction

Water chestnut *(Trapa japonica* Flerov) is a popular aquatic plant distributed in various parts of the world. It has been commercially cultivated for its edible fruits in water bodies on low and flat lands or lakes in India, China and Italy (Daniel et al. 1983, Kumar et al. 1985, Mazumder 1985). In Japan, it is considered a secondary crop (Momoshima and Nakamura 1979) and some species are cultivated in paddy fields in place of rice (Momoshima and Nakamura 1979, Arirna et al. 1999, Hoque et al. 2001).

The fruit contains about 80% starch, 5% protein and significant amounts of vitamins, and is a food source (Khan and Chughtai 1955, Bargale et al. 1987). Fruits are used as a substitute for cereals in the Indian subcontinent, especially during certain important festivals.

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To obtain basic materials for the establishment of large-scale, stable, high yielding chestnut material for planting in the paddy field requires mass production of uniform propagules. Micropropagation is one of the effective means to achieve this goal. However, reports on micropropagation are few (Zhou et al. 1983, Agrawal and Mohan, Ram 1995, Hoque et al. 2001, Hoque and Arima 2002). To our knowledge, there have been no reports on *in vitro* shoot production using various color illuminations on cotyledonary node explants of *T. japonica* Flerov. In this paper, we have compared growth and multiple shoot production using various color illuminations and growth regulators and have reported the best treatment for the mass production of water chestnut nursery plants.

Materials and Methods

Mature, green, bi-spinate, small fiuits of water chestnut *(Trapa japonica* Flerov) were harvested from a water chestnut field at Saga University, Japan and used as the experimental materials. Fruits (50 at a time) were rinsed in distilled water (300 ml) containing three drops of Tween 80 detergent for 15 min. Tween 80 was removed by washing six or seven times in distilled water. The apical end of the fiuit, that partially covers the large cotyledon, plumule, small cotyledon and hypocotyls, was excised (6 - 7 mm^3) and the fiuit was surface-sterilized in a 3% (w/v) sodium hypochlorite solution for 3 min. After three rinses in sterile deionized distilled water, the pericarp was removed and the white embryonal explants (2 - 3 mm long) were excised and cultured on a semi-solid agar 0.6% (w/v) Katayama Co., Japan. MSMA (Huang and Murashige 1976) nutrient media supplemented with 2.7 μM BA, 0.5 μM NAA and 0.5 μM GA_3. Within three to five days of culture under various color illumination (viz. white, blue, mixed, red and infra-red) explants formed cotyledonary nodes early under the mixed color condition. In all treatments, organic nutrients viz. myo-inositol 540.0 μM, pyridoxine 5.4 μM and sucrose 3% were used.

After two weeks of germination on semi-solid media the explants were transferred to culture tubes (55 mm ∇ 100 mm) with 10 ml liquid of the same constitution. For facilitating elongation of shoots and roots, the volume of the liquid medium in the culture vessels was raised to 30 ml. The rooting of shoots was tested by addition of 5.4 μM IBA to the liquid MS under white fluorescent tubes. Rooted plantlets were thoroughly washed to remove all traces of the medium and transferred to plastic pots (50 cm ∇ 30 cm ∇ 22 cm H) filled with sterilized garden soil up to a depth of 5 cm topped with water up to 15 cm. The pots (50 cm ∇ 30 cm ∇ 22 cm H) were kept in the net house under sunlight.

The media were adjusted to pH 5.8 prior to autoc1aving. Cultures were maintained at 28 ± 1°C under a 14 h photoperiod with various color provided by color illumination system (MIL CI000T, Sanyo EC, Japan. Fig. 1). Each experiment was randomized completely, with three replicates per treatment

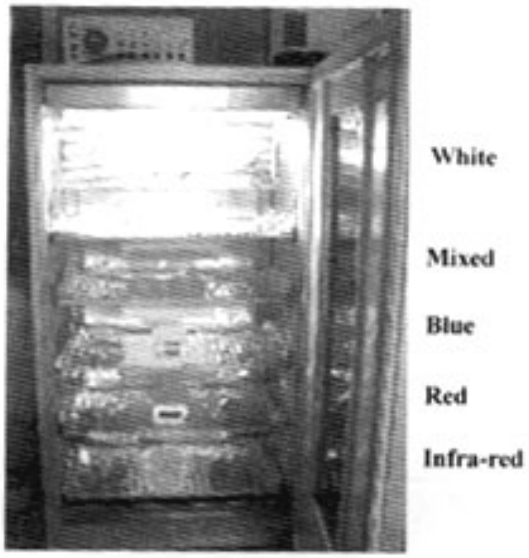

Fig. 1. Incubator for color illumination.

and 18 explants per replicate. Observations were recorded at three weeks interval for shoot multiplication, fresh and dry weight and rooting. The mean values were statistically compared using the Duncan (1955) multiple range test at $p < 0.05$.

Results and Discussion

Effects of various color illuminations on growth morphology of water chestnut seedlings were observed. Among various colors, the growth was maximum under mixed color illumination in every respect followed by red color illumination (Fig. 2). Mixed color illumination also showed higher fresh and dry weight after three weeks of incubation followed by red color (Fig. 3).

The optimum response of explants to mixed color illumination was 95.6% in the liquid media containing MSMA fortified with 2.7 µM BA, 0.5 µM NAA and 0.5 µM GA_3 (Table 1). No significant difference was observed between responses of explants under conditions of white, mixed and red illumination. As a material, cotyledonary nodes were more effective than nodal explants and were

found to have a higher morphogenic potentiality. The morphogenic potentiality of cotyledonary explants has been well documented in other plant species (Detrez et al. 1994, Rajani and Urs 1998, Saba et al. 1999, Hoque et al. 2001).

Fig. 2. Colour illumination effects on fresh and dry weights of tissue culture derived material.

Table 1. Effect of various color illumination on multiple shoot proliferation. Media containing 0.5 mg/l BA, 0.1 mg/l NAA and 0.1 mg/l GA_3 after six weeks growth.

Colour illumination	% of responding explants	No. of shoots per explant
White	95.4 a*	21.6 $\pm$ 0.8 b*
Blue	81.2 b	11.2 $\pm$ 0.2 d
Mix	95.6 a	25.7+1.1a
Red	94.3 a	21.7 + 0.4 b
Infra-red	78.4 b	15.1 $\pm$ 0.4 c

* Comparison of the mean values obtained in treatments were made using Duncan's multiple range test. The values with different letters within a column are significantly different at $p < 0.05$.

Among various colors, shoot production was highest under mixed color regime on the medium containing MSMA supplemented with 2.7 µM BA, 0.5 µM NAA and 0.5 µM GA_3, followed by the red and white lights, respectively. The mixed color illumination produced the maximum 25.7 shoots per plant six weeks after culture, followed by red and white (Table 1). Maybe the mixed color

illumination has a special photosynthetic effect increasing the growth of the explants. The mixed color was the combination of red and blue. All together 100% of illumination, red color was covered 80% illumination with wave length 660 nm and blue color covered 20% illumination with wave 470 nm. Shoot multiplication of water chestnut was higher in liquid medium than solid or semi-solid medium as reported earlier by the authors (Hoque et al. 2001). Growth regulators and nutrient media, used in the present study were also reported earlier. The supplements used in MSMA medium were IAA, sodium phosphate adenine and zip (Huang and Murashige 1976).

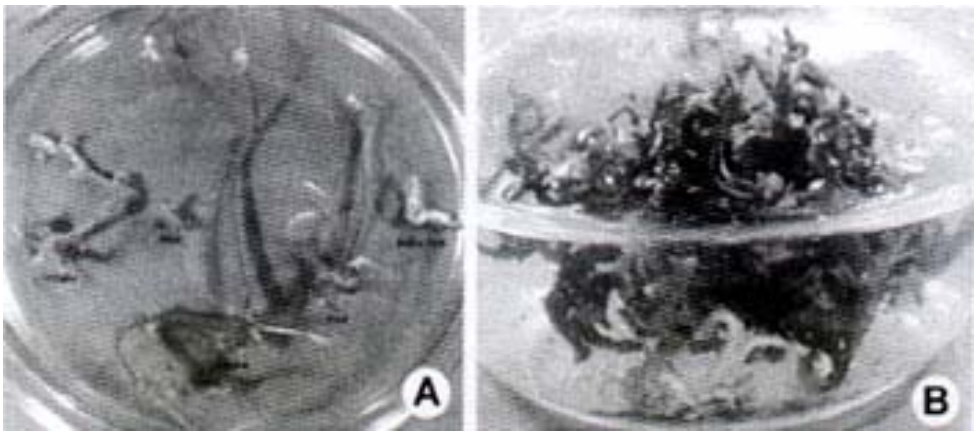

Fig. 3. A. Effect of color illumination on shoot growth after three weeks. B. Shoot growth under mixed color illumination.

For root induction, *in vitro* derived shoots were excised and placed in the liquid rooting medium supplemented with MS salts containing 5.4 μM IBA. In most of the cases, two roots emerged from the node nearest to the transected end of the stem within ten days (data not shown). Roots were not formed in the control medium up to three weeks. Rooted plantlets were transferred to plastic pots filled with sterilized garden soil up to 5 cm, topped with water up to 15 em. Three weeks after culture, new leaves sprouted thereafter they were transplanted to the paddy field where the survival rate was 100%.

The results in this paper under various color illumination regimes showed that the mixed color was the best for early and higher multiple shoot proliferation producing significantly higher fresh and dry weight of the tissue culture derived material.

References

Agrawal A and **Mohan Ram HY** (1995) *In vitro* germination and micropropagation of water chestnut *(Trapa* sp.). Aquatic Bot. **51**: 135-146.

Arima S, Hoque A and **Agarie S** (1999) Comparison of growth and yield perfonnance of several water chestnut species collected from southwestern Japan and middle China. Plant Pro. Sci. **2**: 273-278.

Bargale M, Sawarkar NJ and **Sharma YK** (1987) Mineralogical composition of water chestnut as compared to sweet potato. Indian J. Nutr. Diet. **24** : 7882.

Daniel P, Vajravelu E and **Thiyagaraj JG** (1983) Considerations on *Trapa natans* L. from Peninsular India, J. Econ. Tax. Bot. **4**: 595-601.

Detrez C, Ndiaye S and **Dreyfus B** (1994) *In vitro* regeneration of the tropical multipurpose leguminous tree *Sesbania grandiflora* from cotyledon explant. Plant Cell Rep. **14** : 87-93.

Duncan DB (1955) Multiple range and multiple F tests. Biometrics **11** : 1-42.

Hoque A, Islam R and **Arirna S** (2001) High frequency plant regeneration from cotyledon-derived callus of *Momordica dioica* (Roxb.) Willd. Phytomorphology **50** : 267-272.

Hoque A, Rahman SM, Arima S and **Takagi Y** (2002) Efficient *in vitro* germination and shoot proliferation of chilling-treated water chestnut *(Trapajaponica* Flerov) embryonal explants. In Vitro Cell. Dev. Biol. **37** : 369-374.

Khan WA and **Chughtai ID** (1955) Nutritive value of foodstuffs II - studies in ascorbic acid content of common vegetables. Pakistan J. Sci. Res. **7** : 46-50.

Kumar L, Singh SP and **Pabuja SS** (1985) Studies on vegetative reproduction rate of water hyacinth and water chestnut. Indian J. Agric. Res. **19**: 54-58.

Mazumder BC (1985) Water chestnut-the aquatic fruit, Wid Crps. **37**: 42-44.

Momoshima T and **Nakamura D** (1979) Studies on water chestnut *(T. natans* L.). Bull. Agr. Exp., Saga, Japan **19** : 83-111.

Rajani MS and **Vrs HGVG** (1998) Multiple shoot induction from cotyledonary explants of *Tabebuia* spp. In vitro. Phytomorphology **48** : 207-213.

Saba T, Ghosh M and **Sen SK** (1999) Plant regeneration from cotyledonary explants of jute, *Corchorus capsularis* L. Plant Cell Rep. : 544-548.

Zhou JQ, Zhang ZP, Zhou GY, Liu SZ and **Chen BQ** (1983) Culture of axillary buds of Nanhu Singhara nut. Plant Physiol. Commun. **1** : 31.