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Variation of Callus Induction Through Anther Culture in Water Chestnut (*Trapa* sp.)

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Abstract: Effects of culture media and genotypes of water chestnut (*Trapa* sp.) on the frequency of callus induction from anther culture were investigated. Results revealed that the N_6 media supplemented with 0.5 mg/l BA, 0.5 mg/l NAA and 0.1 mg/l GA_3 were suitable for callus induction. Callus yielded poorly in MS-containing media compared to that of N_6 media. No callus was induced on 2, 4-D containing media. Among the tested 18 genotypes, only 15 genotypes produced calli.

Key Words: Anther culture, callus, water chestnut

Introduction

Water chestnut is an aquatic plant belonging to the Trapaceae family. It is popular for its edible starchy fruits. The fruit contains about 80% starch, 5% protein and a significant amount of vitamins (1,2). It has been commercially cultivated in India, China and Italy for its edible fruits in water bodies on low and plane lands or lakes (3,4,5). Some species are often cultivated in paddy fields in place of rice (6,7,8).

Anther culture is used to produce haploid plants. The technique was first described by Guha and Maheshwari in 1964. They reported direct development of embryos from microspores of *Datura innoxia* by the culture of excised anthers (9). Anther culture has been used for crop improvement and the development of cultivars. This technique has some advantages over the conventional breeding such as its fast regeneration efficiency of desired genotypes (10,11,12). This technique also opened a new avenue of breeding system that covers resistance and quality plant development, shortens the existing breeding cycle with immediate fixation of homozygosity, and increases selection efficiency. With an immense economic importance in anther culture, progress has been made on the cereals (13-18), solanaceous crops (19, 20) and also Brassica (21-23).

The objective of the present investigation was to assess the possibility of anther culture of water chestnut as a method of producing haploids, and in particular, to establish the callus culture and their different genotype effect in a range on culture media.

Materials and Methods

Plant materials

Eighteen cultivars of water chestnut collected from different sources (China I, China II, China III, China IV, China V, China VI, China VII, India I, India II, French, Italy I, Italy II, Kobe - L Japan, Kobe - S Japan, Korean, Israel, Japan M, Japan S) were used for the present experiment. For germination, the collected fruits were immersed in BA 5.0 mg/l plant growth regulator at 15 °C with 12h daylight from 5 to 10 days. Seedlings were then transplanted to water trays and implanted to 15 cm depth on May 13, 2004. The water trays were fertilized with 3.0, 3.4 and 3.0 kg/ha of N, P and K, respectively, as a basal nursing.

From July to September the plants were considered to be at the most appropriate growth stages and were sampled for the experiments. Healthy and vigorously growing flower buds were collected two days before

flowering, where the microspores were determined to be at the uninucleate stage. The exact stage of pollen grain development was also ascertained by dissecting one flower bud from each cultivar, and the anther length was measured using a binocular microscope. Then the anthers were squashed in a drop of acetocarmine to examine developmental stages of the microspores.

Culture media

The callus induction media used for anther culture are shown in Table 1. Upon the completion of medium preparation, the pH was adjusted to 5.8 using 0.2 N NaOH. Gelrite (3.5 mg/l) was used to solidify the media. The medium was sterilized by autoclaving at 121 ℃ for 20 min and after cooling zeatin and GA_3 were added to the medium through filtration (45 μM). The medium was poured into 35 mm Petri dish (10 ml/dish) in a laminar air flow.

Results and Discussion

The genotype proved one of the most important factors affecting organogenesis in the water chestnut. In our study, anthers from more than 18 different genotypes were tested, and callus induction took place in only 15 genotypes, but the growth rates of the calli varied widely. The results of callus induction ability of different genotypes are summarized and presented in Figure 1. The maximum callus induction was achieved in anthers from the Italy (43.9%) genotype, followed by Kobe L (Japan) (43.7%) and French (42.3%) genotypes. Very low frequencies of callus induction were observed in anthers of Korean (16.6%) and China VI (17.8%) genotypes. The calli of these genotypes also showed low growth rates. Variations in callus induction ability among the tested genotypes indicated that difference in response was due to the genotypic difference. The anthers induced callus within 40-60 days after culture and callus induction rate was very low. The calli grew slowly and finally turned brown and rotted. This is believed to have been caused by the lack of appropriate medium. Nutrient media is one of the key factors for callus induction. In the present investigation, we suggest that N_6 basal medium is more suitable than MS basal medium for callus induction (Figure 2). The highest percentage callus (42.3%) was found in N_6 basal nutrient medium fortified with 0.5 mg/l BA, 0.5 mg/l NAA and 0.1 mg/l GA_3, whereas MS basal media with other tested plant growth regulators produced inferior callus frequency (36.1%). Among the 26 tested media, only 10 media persistently produced callus. The highest callus formation was observed in N_6D_1 medium and the lowest in MS_1. The results revealed that low concentration NAA-, BA- and GA_3-containing media were suitable for callus formation (Figure 2). No callus was induced in 2, 4-D containing media.

Table 1. Media used in callus induction experiments from anther as explants of water chestnut.

Media codes	Growth regulators concentration (mg/l)
MSD_1	MS+0.5BA+0.5NAA+0.1GA_3
MSD_2	MS+0.5BA+1.0NAA+0.1GA_3
MSD_3	MS+0.5BA+2.0NAA+0.1GA_3
MSD	MS+1.0 2,4-D
MS_2D	MS+2.0 2,4-D
MS_3D	MS+3.0 2,4-D
MS_5D	MS+5.0 2,4-D
MSK	MS+0.5 KIN+1.0 NAA
MSI	MS+0.5 IAA+1.0 NAA
MSN	MS+2.0 NAA+0.1 GA_3
a	MS+1.0 2ip+2.0 IAA
b	MS+0.2 Zeatin+2.0 IAA
c	MS+1.0 2ip+2.0 IAA
d	MS+1.0 2ip+2.0 IAA
MAC	MS+3.0 2,4-D+0.5 BA+0.5 IAA
N_6D_1	N_6+0.5BA+0.5NAA+0.1 GA_3
N_6D_2	N_6+0.5BA+1.0NAA+0.1 GA_3
N_6D_3	N_6+0.5BA+2.0NAA+0.1 GA_3
N_6D	N_6+1.0 2,4-D
N_62D	N_6+2.0 2,4-D
N_63D	N_6+3.0 2,4-D
N_65D	N_6+5.0 2,4-D
N_6K	N_6+0.5 KIN+ 1.0NAA
N_6I	N_6+0.5IAA+1.0NAA
N_6N	N_6+2.0NAA+0.1 GA_3
X	N_6+1.0 BA+0.5NAA+0.5 GA_3

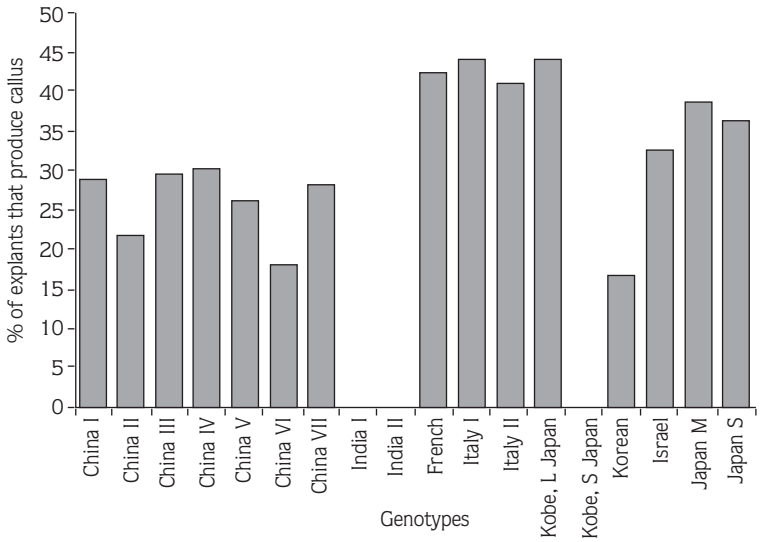

Figure 1. The effect of cultivars on callus formation.

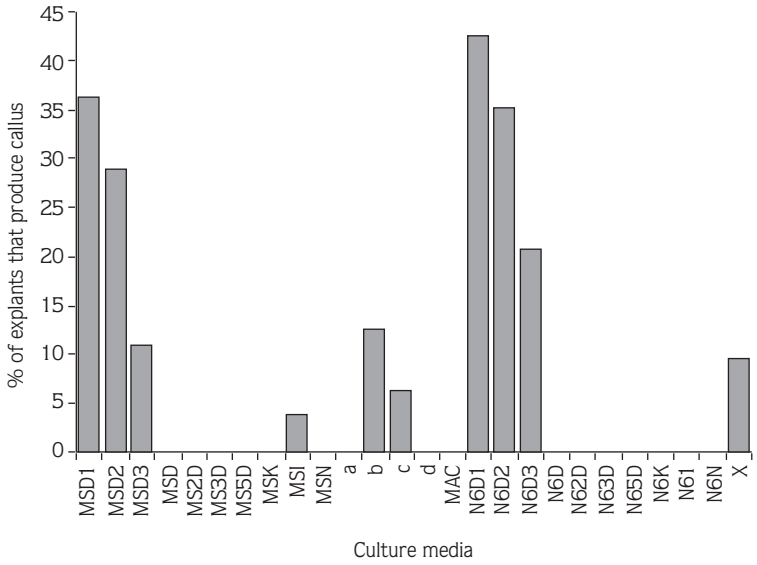

Figure 2. The effect of culture media on callus formation.

The efficiency of calli to organogenesis varied largely among the genotypes. It was also noted that among the 18 genotypes tested, seven genotypes responded to organogenesis. The highest efficiency of organogenesis was achieved after 2nd and 3rd subculture (Kobe L) in MS medium containing 1 mg/l BA and 0.1 mg/l NAA (Figure 3).

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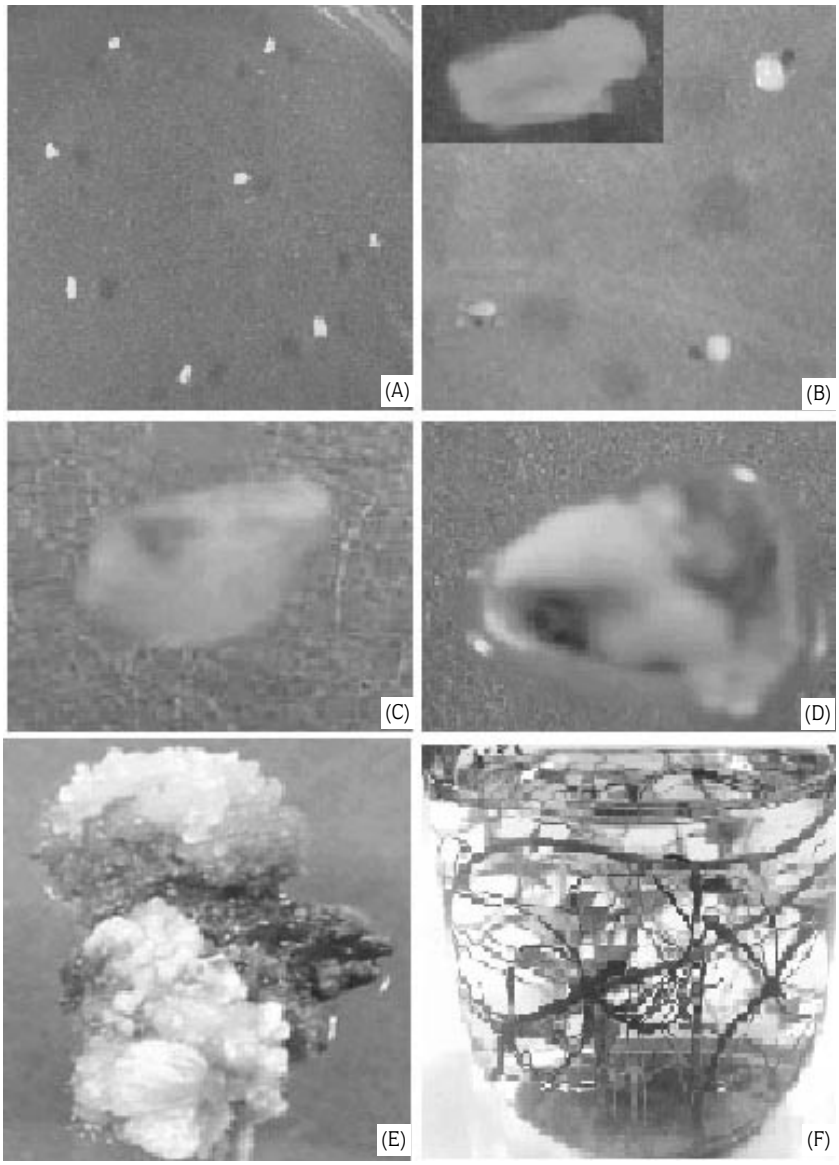

Figure 3. Summary of anther culture. (A) Anther after 3 days of culture, (B) Callus initiation from anther after 2 weeks, (C) Development of callus from anther after 3 weeks of culture, (D) Development of callus from anther after 4 weeks of culture, (E) Organogenesis from anther-derived calli after 8 weeks of culture, (F) Rooting of the shoots.

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