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A SIMPLIFIED METHOD OF EMBRYO CULTURE IN RICE OF *ORYZA SATIVA* L.

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In addition to fundamental embryogenesis studies, embryo culture has been used as a means to rescue inviable embryos, reduce breeding passages, overcome dormancy (Raghavan, 1977) and even for the production of haploids in barley (Jensen, 1977) and hexaploid wheat (Barclay, 1975). In recent years, immature embryos of different species have also been extensively used as donor tissue in the induction of callus (Green and Philips, 1975; Cummings *et al.*, 1976; Gamborg *et al.*, 1977; Dale and Deambrogio, 1979; Shimada and Yamada, 1979; Springer *et al.*, 1979; Sharma *et al.*, 1980 and 1981), partly because of its ease in regeneration into plantlets. In cereals, e.g. rice, the excision of embryo requires and the removal of the palea and lemma become difficult with matured caryopsis. Then, the ovary needs to be dissected under a microscope. The process is laborious and time consuming. In addition, even a well-trained worker may unwittingly cause damage to the embryo through the handing of blades and needles.

We have developed a simplified procedure whereby embryos can be obtained sterile and intact without a microscope and other dissecting equipments. The process is fast and efficient. It is especially suited for immature embryos in the late milk to soft dough stage, about 10–18 days after pollination, and may be applicable for embryos as small as 0.5 mm in length.

Basically, the method involves making a slight clip at the very basal end of the grain and squeezing its sides with fingers. Through this simple manipulation, embryo embedded in the ovular sap can be easily forced out through the cut end. Visible to the naked eye, they are ready for transfer to the nutrient medium with a loop. Using this method, we have successfully excised more than 200 embryos from two rice varieties Tainan 5 and Tainung 67. Contamination was less than 1%, and 95% of the embryos cultured developed into healthy seedlings. Protocol of the method is as follows:

—Detach panicles with spikelets in the desired developmental stage.
—Discard empty and appearently diseased spikelets.
—Wash panicles profusely in running tap water for 10 minutes.
—Immerse panicles in 70% alcohol for 1–2 minutes.
—Remove excess alcohol with absorbent cotton.
—Transfer panicles to 3.5% sodium hypochloride solution to which 2–3 drops of Tween-20 have been added. Let it stand for 3–4 hours (Fig. 1).
—Wash panicles throughoutly in sterile water.
—Detach spikelets individually and place them on a flamed aluminum foil (12×8 cm) in a laminar flow bench.

Table 1. *Composition of media A and SR*

	Medium A (mg/l) inducing calli	Medium SR (mg/l) regeneration
Macro components		
KNO_3	2,275	2,730
NH_4NO_3	600	720
$CaCl_2 \cdot 2H_2O$	221.08	352.8
KH_2PO_4	136	179.52
$MgSO_4 \cdot 7H_2O$	185	296
Micro components		
Na_2EDTA	37.3	37.3
$FeSO_4 \cdot 7H_2O$	27.8	27.8
KI	0.75	0.75
$MnSO_4 \cdot 5H_2O$	10	10
H_3BO_3	3.0	3.0
$ZnSO_4 \cdot 7H_2O$	2.0	2.0
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25
$CuSO_4 \cdot 5H_2O$	0.025	0.025
$CoCl_2 \cdot 6H_2O$	0.025	0.025
Vitamins and hormons		
Nicotinic acid	1.0	1.0
Thiamine HCl	10.0	10.0
Pyridorine HCl	1.0	1.0
Myo-isoitol	100	100
NAA	—	0.2
2, 4—D	2-4	—
*Kinetin	2.0	0.2
Protein hydrolysates		
Casein hydrolysate	—	250
Sucrose	30,000	60,000
Agar	9,000	9,000

—Clip spikelets 2-3 mm above the pedicel end with a pair of sterilized scissors (Fig. 2).

—Using one's thumb and forefinger and taking care not to touch the cut end, slowly squeeze the embryo out of the floret and transfer it to a nutrient medium with a loop (Fig. 3)

Cultured embryos start to develop into seedlings 2-3 days later under a regime of 16/8 hours light/dark photoperiod, 6,000 luxes at 26±1°C (Fig. 4). with those young embryos, preculturing in the dark for a few days may be helpful in preventing precocious germination which results in weak and slender seedlings.

Two media satisfactorily used in our laboratory are also listed (Table 1) Medium A is for the induction and proliforation of callus, and medium SR (containing sucrose for regeneration) for growing immature embryos of sizes more than 0.6 mm in length. The addition of phytohormones α-Napthalene acetic acid (NAA) and kinetin to the medium SR is unnecessary but beneficial, especially for younger embryos.

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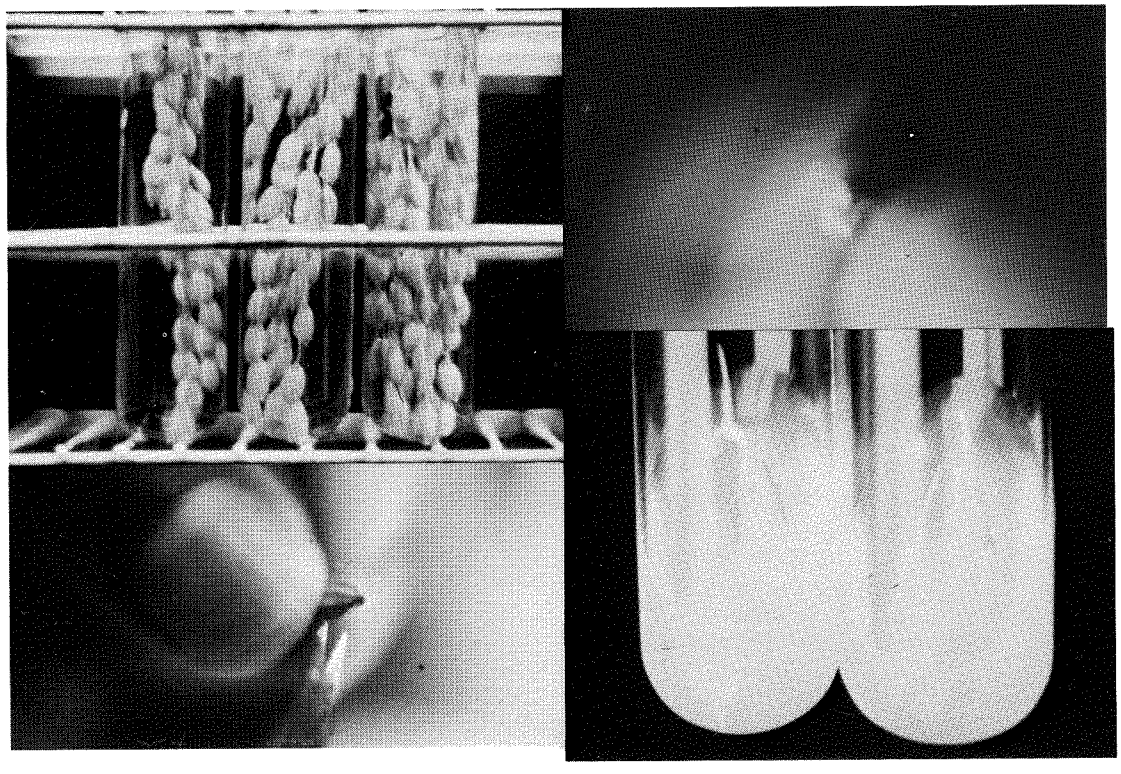

Fig. 1. Panicles under sterilization in 3.5% sodium hypochlorite solution.

Fig. 2. Spikelet being clipped at the pedical end.

Fig. 3. Embryo obtained by squeezing the sides of the clipped spikelet.

Fig. 4. Very young seedlings developed from cultured embryos of about 12 days old.

水稻胚之簡易接種

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本文介紹水稻胚接種的一種簡易方法。使用剪刀在乳熟期的穀粒基部剪一個小洞，用手指輕輕將胚及胚乳由小洞中擠出。以解剖針挑起，置於培養基中培養。以臺南5號及臺農67號爲材料，成功地挑出200個以上的胚，置於培養基中生長，僅有1%以下的胚受汚染。95%以上的胚都能生長成强健的小苗。