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Micropropagation of Maize Plant through Seed Culture in vitro

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Micropropagation of dicots in vitro can be achieved through both unorganized systems such as callus and suspension cultured cells, and organized systems such as shoot tips, meristems (vegetative organs), seeds and embryos (reproductive organs).[1,2] However, no report has yet dealt with micropropagation of the maize plant, a monocot, though plant regeneration from callus and suspension culture has been reported.[3]

The authors have studied plant micropropagation through culture of reproductive organs, such as embryos and seeds, of several other species.[4] In this communication, mass propagation of maize by multiple shoot induction from seeds, successive shoot multiplication and rooting from excised shoots are described.

Maize seeds (*Zea mays* L. cv. Early King) were purchased from Northrup King Seeds (Minneapolis, Minn.). Maize seeds, cv. Hannybantam (Sakata Seeds, Yokohama, Japan) gave similar results to those of Early King. The experimental conditions were similar to those described previously.[4,5] Seeds and explants were cultured in the same modified Murashige and Skoog's medium as that described previously except that the medium contained White's organic nutrients and that hormonal constituents were altered. Seeds and explants were cultured at 27°C under an irradiance of 3,000 lux (16 hr light and 8 hr dark).

Multiple shoot induction from seeds

The combination of several types of plant hormones are usually examined to induce multiple shoots from organized systems. In the preceding experiments, multiple shoots were induced from various dicotyledonous seeds by cytokinin alone.[4,5] In the present experiment, maize seeds were cultured for 4 weeks in the medium containing a single cytokinin, i.e. 6-benzylaminopurine (BAP), kinetin or N^6-(2-isopentenyl) adenine (2 iP) at various concentrations (0, 0.05, 0.25, 0.5, 5.0 and 50 μM).

BAP induced multiple shoot buds while kinetin and 2 iP did not induce multiple shoot buds at the concentration used. Kinetin and 2 iP at higher concentration (150 μM) could also not induce multiple shoot buds. Multiple shoot buds were only observed in the presence of 5 μM and 50 μM of BAP. The ratio of seeds forming multiple shoot buds to those forming single shoot increased with increasing BAP concentration. BAP 50 μM medium was adapted to induce multiple shoot buds. However, the greater the increase in BAP concentration, the greater the inhibition of shoot bud growth. In the 50 μM BAP medium, multiple shoot bud growth was poor in the initial culture. To accelerate shoot bud growth, seeds were transferred to a lower BAP medium, for instance 5 μM BAP medium, and cultured for another 3 weeks. The multiple shoot buds grew well at the end of the culture (**Fig. 1**). The multiple shoot buds excised from these cultures were used in the shoot multiplication process.

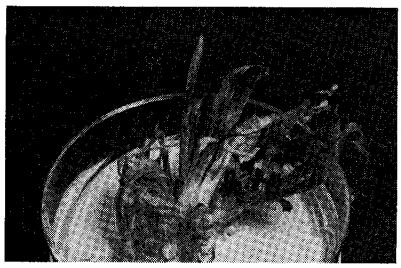

Fig. 1. Multiple shoots from maize seeds in vitro. Seeds were cultured in 50 μM BAP medium for 4 weeks and subsequently cultured in medium containing 5 μM BAP for 3 weeks.

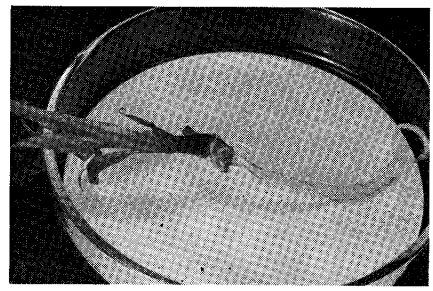

Fig. 2. Rooting from maize excised single shoots. Shoots were excised from cultures obtained as in **Fig. 1** and cultured in medium containing 0.5 μM IBA for 3 weeks.

Successive shoot multiplication

Usually multiple shoot induction from single shoots is attempted to establish successive shoot multiplication of dicots. In the present experiment, trials were conducted with clumps consisting of several multiple shoot buds. Every clump excised from each of the above seed cultures was separated into 3 small clumps. The small clumps were cultured in the medium containing a constant concentration of BAP (0.5 μM) and various concentrations of indolebutylic acid (IBA, 0–1 μM). The combination of BAP 0.5 μM and IBA 0.025 μM gave good continuous multiplication and growth of shoot buds. Under these conditions, clumps multiplied continuously about three times in a 4 week period. One can calculate that $3^{365/28} \fallingdotseq 3^{13} = 1,594,323$ clumps could be obtained from a single clump in a year.

The shoots propagated here were used for the following rooting experiment.

Plant regeneration by rooting

Single shoots longer than 3 cm in length excised from multiple shoots were cultured in a medium containing different concentrations of IBA (0–5 μM). At each concentration, about 80% of the shoots examined developed roots (**Fig. 2**). Clumps also rooted at the same extent in the same range of IBA concentration as mentioned in the rooting experiment of single shoots.

General consideration

Multiple shoot bud induction from dicotyledonous tissues or organs may be explainable by reduction of apical dominance or induction of meristematic regions by cytokinin. Usually each maize plant has only one culm in the field. However, it is known that a maize plant has potentially tiller buds.[6] In the present experiment, tiller bud proliferation was observed in some cases. To date the former explanation as for dicots seems to be attractive to explain the phenomenon of multiple shoot bud induction in maize seedlings and clumps. However, further study is necessary to determine more accurately the mechanism of this phenomenon in maize, a monocot.

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≪和文要旨≫

トウモロコシ種子からの大量迅速育苗について

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BAP を含む培地でトウモロコシ種子からマルチプルシュートを誘導できた．しかし，キネチンあるいは 2 iP 培地では誘導できなかった．生じたマルチプルシュートの連続的増殖は可能で，計算上年に約 160 万倍の増殖が可能である．単一シュートから植物体を復原し得た．マルチプルシュートの起源について考察した．