Short Communication

Propagation of almond by shoot tip culture

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Shoot tips 0.5 mm long were taken from actively growing almond (*Prunus amygdalus* Batsch cv. hardy) shoots. These were cultured on the modified 1/2 MS medium (UEMATSU and AKIHAMA 1987) supplemented with 6-benzyladenin (6-BA) or N-(2-chloro-4-pyridyl)-N-phenylurea (4 PU, Kyowahakko Kogyo K. K.). About 70 to 80% of shoots survived on the paper-wick liquid medium containing 2.0 or 4.0 mg/l 4 PU. Growing shoots were transferred to the solid multiplying medium containing 2 g/l gerlite and 0.5 mg/l 6-BA. These shoots were subcultured on the same fresh medium at forty days intervals. After nine months of culture, the multiplication rate was 6.6-fold per forty days. The longest shoot reached to 3.8 cm. Within a month after transferring to the rooting medium containing 0.5 mg/l naphthalene acetic acid (NAA), root induction occurred.

KEY WORDS : *Prunus amygdalus*, almond, shoot tip culture, 4 PU.

Introduction

Shoot tip culture in fruit trees was started in 1960s for the purpose of micropropagation and virus elimination. There have been some reports of shoot tip culture for successful micropropagation in some *Prunus* rootstocks (JONES and HOPGOOD 1979, HAMMERSCHLAG 1982, MILLER *et al.* 1982), sweetcherry and sourcherry (SNIR 1982, 1983).

Shoot tip culture of almond has been reported using dormant buds collected from October to February (TABACHNIK and KESTER 1977), without mentioning the size of shoot tips cultured. There was, however, little growth of shoots on the paper wicks in their experiment.

In the present study, shoot tips 0.5 mm long were taken from actively growing shoots and high survival rate on the paper-wick medium containing 4 PU was observed. Here we report the conditions for shoot multiplication and root induction. Since the original size of shoot tip was as small as 0.5 mm, this method can be used for virus elimination.

Materials and Methods

Shoot tips were obtained from actively growing shoots of almond (*Prunus amygdalus* Batsch cv. hardy) on 10th of May, 1986. After removing leaves, shoots reduced to 1.5~2.0 cm in length. Cut shoots were washed in neutral detergent and rinsed in running tap water. These were sterilized in soaking in 70% ethanol for 3 min. and immersing in 0.5% sodium hypochlorite solution for 5 min., and were rinsed twice in sterile water. Shoot tips of 0.5 mm in length were taken from cut shoots under the stereo microscope, and were placed on the medium.

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The basic medium was composed of modified 1/2 MS medium (UEMATSU and AKIHAMA 1987) in which inorganic salts other than iron were reduced to a half of MS (MURASHIGE and SKOOG 1962) but the other elements were kept at full strength of the MS medium. To establish cultures, 2.0 mg/l 6-BA, 2.0 or 4.0 mg/l 4 PU was added to the basic medium supplemented with 30 g/l sucrose, 2 g/l activated charcoal and 1 g/l agar. Five milliliter of liquid medium poured into a test tube and a filter paper wick was applied on the medium.

After culturing forty days on this establishment medium, shoots were transferred to the multiplying medium. The multiplying medium contained 0.5 mg/l 6-BA, 30 g/l sucrose and 2 g/l gerlite in addition to the modified 1/2 MS medium. Shoots were subcultured every forty days to the fresh multiplying medium containing 0.5 mg/l 6-BA.

Some of the newly induced shoots were transferred to various rooting media. These rooting media were composed of the modified 1/2 MS medium with 30 g/l sucrose and were variously supplemented by 0.1 or 0.5 mg/l NAA or 0.1 or 0.5 mg/l indolebutyric acid (IBA). They were solidified with 2 g/l gerlite.

The cultures were maintained at 23°C with 14 hr illumination under the fluorescent light. For the root induction, the culture vessel was put on the black paper and kept under the condition stated above.

Results

On the establishment medium, the shoots turned to light green and the leaves elongated. Brown callus was occasionally formed at the bottom end of a shoot tip. After forty days of culture, the survival rate of shoot tips was the highest on the establishment medium with 4.0 mg/l 4 PU, 80%, and the second on the one with 2.0 mg/l 4 PU, 70% (Table 1, Fig. 1). Survival rate was the lowest, 40%, on the establishment medium with 2.0 mg/l 6-BA. Same results were obtained from an experiment in 1987.

The growth and multiplication of the elongated shoots were stable after transferring to the multiplying medium with 0.5 mg/l 6-BA. Shoots were maintained by subculturing every forty days on the multiplying medium with 0.5 mg/l 6-BA. Shoot multiplication observed was due to axillary bud elongation. An adventitious bud formation did not occurred. Shoot multiplication rate became higher accord-

Table 1. Survival rates of almond shoot tips cultured on establishment medium

cytokinin	conc. (mg/l)	No. of shoot tips placed	No. of shoots survived ¹⁾
4 PU	2.0	10	7
4 PU	4.0	10	8
6-BA	2.0	10	4

1) Elongated shoot was counted.

Table 2. Distribution of shoot length obtained from a subculture passage

range of shoot length (mm)	No. of shoots	range of shoot length (mm)	No. of shoots
0~5	0	26~30	4
6~10	13	31~35	0
11~15	16	36~40	2
16~20	15	41~	0
21~25	7		

Shoots were subcultured about 5 months on the modified 1/2 MS medium containing 0.5 mg/l 6-BA. Shoot length was measured 30 days after transferring to the fresh medium.

maximum : 38 mm

mean : 15 mm

- Fig. 1. Shoot cultured for 3 weeks on the paper-wick medium containing 2.0 mg/l 4PU.
 Fig. 2. Shoot multiplication obtained from a passage of subculture on the multiplying medium containing 0.5 mg/l 6-BA.
 Fig. 3. Shoot elongation on the multiplying medium containing 0.5 mg/l 6-BA.
 Fig. 4. Root induction on the medium containing 0.5 mg/l NAA.

ing to the subculturing. After nine months culture each shoot produced two to eighteen shoots through one passage of subculture. Shoot multiplication rate reached to 6.6-fold (Fig. 2). Length of shoots became a maximum of 3.8 cm, and an average was 1.5 cm (Table 2, Fig. 3).

When newly multiplied and elongated shoots were transferred to the rooting medium containing 0.5 mg/l NAA, root induction occurred within 30 days (Fig. 4). On all the other rooting media containing 0.1 or 0.5 mg/l IBA or 0.1 mg/l NAA, root induction did not occur.

Discussion

There have been some reports on shoot tip culture in *Prunus*. Cytokinin was indispensable for the establishment of culture. So far in the previous reports, 6-BA was used as a cytokinin (TABACHNIK and KESTER 1977, JONES and HOPGOOD 1979, etc.), with an only exception (SNIR 1984) using 2iP. Previously we reported, however, that 4PU was effective for the shoot tip culture in plum (UEMATSU and AKIHAMA 1987). This seems to be the first report on 4PU applied in shoot tip culture. In the

present study, it became clear that 4PU was more effective than 6-BA for the establishment of almond shoot tip culture.

It seems that the effectiveness of 4PU on shoot tip culture was due to its higher activity as cytokinin than other natural or synthetic cytokinins. In tobacco callus culture, 4PU was revealed to have strong activity for shoot formation (OKAMOTO *et al.* 1978). Each of natural or synthetic cytokinin has a purine ring, but 4PU is a diphenylurea compound without a purine ring. The difference in structure of 4PU and function possibly caused the difference in effectiveness. Now, further investigation on the effect of 4PU is continued in our laboratory with other fruit crops in which shoot tip culture was difficult or not successful.

In the present study, shoot multiplication and root induction were demonstrated from small shoot tips of 0.5 mm in length taken from actively growing almond shoots. This method could be used for micropropagation and virus elimination.

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茎頂培養によるアーモンドの増殖

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核果類の茎頂培養は 1960 年代に台木の大量増殖やウイルスフリー化を目的として始められた。アーモンドにおいても休眠芽を用いた茎頂培養が報告されているが、外植片として用いる茎頂の大きさが明らかにされていない (TABACHNIK and KESTER, 1977)。ウイルスフリー化を目的とする場合には茎頂を可及的に小さく切り取り培養することが重要である。筆者らは伸長中のアーモンドの新梢から茎頂部分 0.5 mm を取り出し培養したところ、芽条の増殖と発根に成功した。基本培地は MS 培地の鉄を除く無機成分を規定の 1/2 濃度にした修正 1/2 MS 培地 (UEMATSU and AKIHAMA, 1987) を用いた。これに 4 PU 2.0~4.0 mg/l, 活性炭 2.0 g/l を添加し、ペーパーウィック培地とした。40 日間初代培養した時、70~80% の高い生存率を示した。初代培養後、基本培地に 6-BA 0.5 mg/l を添加し、ゲルライト 2 g/l で固形化した増殖培地で継代培養をくり返した。40 日ごとに継代を重ねると芽条の増殖率が高まり、9 ヶ月めには 1 回の継代培養で 1 個の茎葉から 2~18 個の新しい芽条が形成され、増殖率は 6.6 倍に達した (Fig. 2)。また茎葉の節間も伸長し最大で 3.8 cm, 平均 1.5cm だった (Table 2, Fig. 3)。増殖、伸長した茎葉を切り分けて NAA 0.5 mg/l を含む培地に移植すると根が形成された。本研究の結果、アーモンドで 0.5 mm の茎頂を培養して芽条の増殖、発根に成功したので、今後この方法を用いることによりウイルスフリー化や自根苗の増殖が可能になると考える。

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