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Short Communication

High-efficiency regeneration of peanut (*Arachis hypogaea* L.) plants from leaf discs

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A high-efficiency regeneration system for peanut plants was established. The regeneration frequency of leaf discs reached 40.9% on Murashige and Skoog medium supplemented with 0.5 mg l^{-1} naphthylacetic acid and 0.5 mg l^{-1} thidiazuron. The regenerated shoots elongated, developed roots and produced seeds. This procedure was highly efficient and is feasible for the genetic transformation of peanuts.

Key words: Peanut, regeneration, high efficiency.

INTRODUCTION

Peanut, one of the most important oil crops, is a good source of oils, proteins, calories and vitamins. Peanuts are also a safe alternative to reduce hunger in Asia, Africa and Latin America. Global peanut production has reached 34.42 million metric tons, with China producing the highest amount of peanuts any country can produce. Peanut yields are substantially reduced because of the damage caused by subterranean insects and bacterial and fungal diseases (Vargas et al., 2008). The recalcitrance of peanuts to tissue regeneration and genetic transformation impedes the development of genetically modified approaches for pest and disease control.

Several exogenous genes have been introduced into peanuts by particle bombardment (Chu et al., 2008) or Agrobacterium-mediated transformation (Bhatnagar et al., 2010), but these genetic transformation approaches are time-consuming and labor-intensive, and a vast amount of explants are needed due to the low frequency of regeneration in peanuts. The successful exploitation of *in vitro* techniques in peanuts depends on the establishment of efficient regeneration systems. Leaflets are the most widely used explants in peanut tissue culture. Several other types of explants, such as cotyledonary nodes (Srinivasan et al., 2010), epicotyls, hypocotyls (Marion et al., 2008), axillary meristems (Singh and Hazra, 2009) and cotyledons (Bhatnagar et al., 2010; Tiwari and Tuli, 2008), have also been used in peanut regeneration systems. Although, great efforts have been made to enhance the frequency of regeneration in peanuts, it was still difficult to obtain a sufficient number of explants in a short period of time. It even takes 4 to 6 months for explants to regenerate and recover from selection (Bhatnagar et al., 2010).

The proper combination of hormones could induce the proliferation of meristematic tissues and convert the explants into complete plants. In this study, a reproducible and high-efficiency regeneration system for peanuts was established using young leaves in medium supplemented with naphthylacetic acid and thidiazuron.

MATERIAL AND METHODS

Mature peanut seeds of *Baisha1016* without shells were sterilized by incubating them in 75% ethanol for 1 min and then in mercuric chloride for 4 min. The seeds were washed three times with sterilized water. The two cotyledons of one seed were separated using a scalpel, and the one with the intact embryo was put on basal MS medium (Murashige and Skoog, 1962) supplemented with 30 g l^{-1} sucrose and 7.5 g l^{-1} agar. Plants were kept in the growth chamber with a 14 h/35 µmol m^{-2} s^{-1} photoperiod at 26 to 28°C. The leaf margin of six-day-old seedlings was cut off, and the leaf discs were put on different media to develop shoots (Figure 1a).

To assess the optimal shoot induction medium, basal MS medium was supplemented with different concentrations of naphthylacetic acid (NAA) (0, 0.5, 1 or 2 mg l^{-1}) and thidiazuron (0, 0.2, 0.5 or 1 mg l^{-1}). Sixteen medium combinations based on MS

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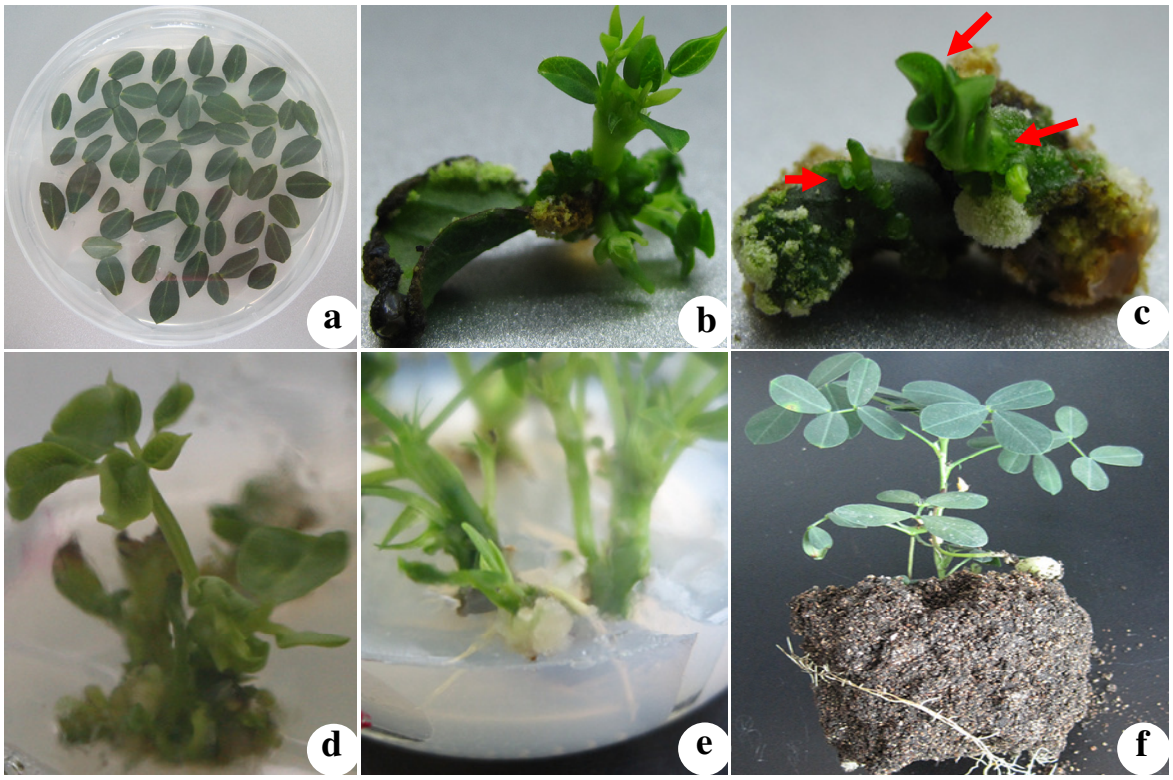

Figure 1. Regeneration of peanuts from leaf discs. A, Leaf discs; b, shoots regenerated from a leaf disc; c, induced buds; d, shoots elongation; e, rooting; f, transplantation of seedling and seed-setting.

medium were prepared and transferred to plastic Petri dishes. Then, 35 leaf discs were selected for each medium. The number of buds was counted after 40 days. The rate of inducing buds was calculated by dividing the number of explants that developed induced buds by the total number of explants.

To determine the optimal shoot elongation medium, basal MS medium was supplemented with different concentrations of 6-benzylaminopurine (6-BA) (4 or 8 mg l^{-1}) and naphthylacetic acid (0.5 or 1 mg l^{-1}). Four medium combinations based on MS medium were prepared, and then 30 shoots were selected for each medium. The number of elongating shoots was counted after 20 days. The percentage of shoots elongating was calculated by dividing the number of the elongating shoots by the total number of explants.

Regenerated shoots were transferred to root induction medium, which consisted of MS medium supplemented with 0.5 mg l^{-1} naphthylacetic acid. Shoots with robust roots were transplanted to pots (11 cm high and 13 cm in diameter) with nutritional soil and vermiculite mixture (volume ratio 2:1).

RESULTS

The sterilized peanut embryos developed into seedlings with 8 or 12 leaves after 6 days. The leaf margins were cut off, and the leaf discs were put on media containing various combinations of hormones to determine which combination is the most effective to induce buds. The result shows that buds were regenerated from discs on some medium combinations, but the frequency of bud formation was below 15% for all media, except MS medium supplemented with 0.5 mg l^{-1} naphthylacetic acid and 0.5 mg l^{-1} thidiazuron (labeled as L medium), for which the frequency of bud formation was 31.4%. Buds were visible on leaf discs, 20 days after placement on L medium (Figure 1c), and the induced buds developed into shoots in the next 20 days (Figure 1b). The average rate of induced buds reached 40.9% based on the results of three independent experiments on L medium (Table 1). Regenerated buds were subcultured onto MS media supplemented with 6-benzylaminopurine and naphthylacetic acid; otherwise, the explants would develop abnormal adventitious buds. The results show that the frequency of shoot elongation ranged from 26.7 to 83.3% (Table 2). Based on three independent experiments, 79% of shoots (Table 2) grew about 2 more centimeters in 20 days on MS medium supplemented with 8 mg l^{-1} 6-benzylaminopurine and 0.5 mg l^{-1} naphthylacetic acid (Figure 1d). These shoots were able to develop roots on MS medium supplemented with 0.5 mg l^{-1} naphthylacetic acid (Figure 1e). Then, the seedlings were transplanted to pots, and the whole plant became stronger in the greenhouse. Furthermore, these plants had a normal ability to produce seeds (Figure 1f).

DISCUSSION

Prior to this study, the highest reported frequency of

Table 1. Percentage of induced buds on L medium.

Repeat	Number of explant	Number of shoot explant	Percentage of shoot explant (%)	
1	35	11	31.4	
2	35	16	45.7	40.9±8.3
3	35	16	45.7	

Table 2. Effect of 6-benzylaminopurine and naphthylacetic acid on the elongation of shoots in peanuts.

6-BA (mg l^{-1})	NAA (mg l^{-1})	Number of explant	Number of elongating shoot	Percentage of elongating shoot (%)	
4	0.5	30	8	26.7	
4	1	30	13	43.3	
8	1	30	6	20.0	
		30	25	83.3	
8	0.5	30	24	80.0	79.7±3.7
		29	22	75.9	

induced shoots was 34.7% (Akasaka et al., 2000), which was obtained by growing peanut leaves on thidiazuron-containing medium. In this study, the optimal medium, containing naphthylacetic acid and thidiazuron, performed better than the media studied before. In the initial experiment, 16 combinations of 6-benzylaminopurine (0, 5, 7.5 or 10 mg l^{-1}), naphthylacetic acid and thidiazuron were used to investigate the effects on bud formation. Most explants developed abnormal enlarged tissue. The results indicate that excessively high concentrations of cytokinins have side-effects on shoot organogenesis, although, a high cytokinin-to-auxin ratio has been shown to lead to shoot regeneration (Kakani et al., 2009).

A fast and efficient regeneration system is a prerequisite for the genetic transformation of peanuts and the improvement of peanut production and quality through molecular breeding. Explants did not exhibit good regeneration ability on medium containing a single hormone, and a proper cytokinin-to-auxin ratio is important during organism development. Peanut tissue culture has been previously investigated in some studies, and several explants were used. However, the long period of tissue culture and the low frequency of regeneration made the genetic transformation of peanuts to be significant for undertaking. In this study, 40.9% of explants developed multiple buds on MS medium supplemented with 0.5 mg l^{-1} naphthylacetic acid and 0.5 mg l^{-1} thidiazuron. This procedure improved the regeneration efficiency and obviated the need for a laborious regeneration process.

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