Effect of plant growth regulators on direct shoot regeneration of wheat (*Triticum aestivum*)

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Abstract

A new, simple and efficient method was developed for multiple shoot regeneration of wheat. The effects of different explants and various combinations of plant growth regulators on direct shoot regeneration of wheat were investigated. This method yielded a number of shoots within a short period of time. Shoot proliferation and elongation was achieved on shoot regeneration medium without any subculture. Direct shoot regeneration is important since fewer somaclonal variations are likely to arise in indirect regeneration method. Among different explants and different combination of 2,4-D, IAA, NAA and BAP tested, embryo explants cultured in MS medium supplemented with 1mg/l BAP, 0.2mg/l 2,4-D and 0.2mg/l IAA and excised embryo explants cultured in MS medium supplemented with 0.2 mg/l 2,4-D and 2 mg/l BAP resulted in the most efficient direct shoot regeneration and produced a maximum of 7 shoots per explants. Also important for shoot regeneration was the effect of the 4-week period of exposure to darkness followed by light exposure. Plantlets were successfully transferred to rooting medium.

Media summary

A new and interesting method for direct shoot regeneration of wheat with embryo and excised embryo explants reported by Iranian biotechnologists for the first time.

Key words

Wheat, direct shoot regeneration, tissue culture, embryo, excised embryo

Introduction

Wheat is a member of the family Poaceae that includes major cereal crops of the world such as maize, wheat and rice. Among the food crops, wheat is one of the most abundant sources of energy and proteins for the world population. Ninety-five percent of wheat grown today is of the hexaploid type, used for the preparation of bread and other baked products. Wheat is characterized by a large genome size (approximately 17000 Mb), thus making the improvement process by any method genetically challenging (Debasis Patnaik, 2001). However, as is the case for many members of the monocot species, not all wheat species respond to the same conditions of in vitro culture for plant regeneration. All of the approaches have focused mainly on the establishment of a regeneration system via callus induction, that has proven to be a multi-step procedure, which requires a long time interval for the development of whole plants. There is also a risk of somaclonal variation when the procedure involves a callus phase. Therefore, a rapid multiplication by direct plant regeneration in large quantities by micropropagation is needed (D.Nhut, B. Lee, and T. Van, 2000).

In this paper by simply manipulating the concentrations of BAP, 2,4-D, IAA and NAA in the culture medium, we describe a method for rapidly obtaining whole plants without the subculture of wheat using embryo and excised embryo explants. The effects of dark/light condition for improving direct shoot regeneration are presented.

Material and Methods

Plant materials

Wheat seed of cultivars Tajan and Azadi were used for this experiment. Seeds were surface sterilized by immersion in 70% ethanol for 1.5 min and 2.5% sodium hypochlorite for 10 min and were washed with sterile water three times, each time for 5 min. Sterilized seeds were placed in 4? c for 24-48 hours. The pH of medium was adjusted to 5.8 before autoclaving. The explants were incubated at 25?1?c in dark or light condition for 4 weeks.

Culture condition

Four types of explants were used for this experiment. Stem node, scutellum, embryo and excised embryo. Between different kinds of explants stem node and scutellum didn't respond to regeneration. The effect of different combinations of 2,4-D, IAA, NAA and BAP were studied. To examine the effect of exposure to the dark, the explants were placed in the dark for 4 weeks before transferring to light conditions.

Shoot development and rooting

Regenerated shoots from the embryo and excised embryo explants were transferred to MS medium supplemented with 0.5mg/l IAA for vigorous and phenotypically normal shoot development and rooting.

Results

In control experiments, where explants were cultured on a medium without 2,4-D, callus formation was not observed. The capacity to form direct shoot regeneration increased with an increase in BAP concentration up to 2mg/l. With an increase in 2,4-D concentration up to 2mg/l callus formation increased and direct shoot regeneration decreased. Results showed that lower concentrations of auxins are necessary for direct shoot regeneration and formation of multiple shoots. Best results were obtained on MS medium supplemented with 0.2mg/l 2,4-D, 1mg/l BAP and 0.2mg/l IAA from embryo explants. This optimal concentration giving rise to the highest number of direct shoots formed from embryo explants were kept in the dark for 4 weeks and then exposed to light (5 shoots per embryo). The highest number of direct shoots from excised embryo explants obtained on MS medium contained 0.2mg/l 2,4-D and 2mg/l BAP (7 shoots per excised embryo). Explants that were kept in the dark for a period of 4 weeks before exposure to light, showed better direct regeneration.

Conclusions

A new method was reported for the direct shoot regeneration of wheat without subculture on regeneration medium. The positive effect of auxins, especially 2,4-D, may be due to the fact that the stimulated auxin may have changed the balance of endogenous growth regulators at the germination stage, and thus enhanced direct shoot regeneration induction. In the present study, results have shown that dark/light conditions are also effective for direct shoot regeneration. Darkness was generally observed to stimulate more direct shoots than light condition. The effect of light can be interpreted as acting on metabolism and sugar uptake.

In conclusion, direct shoot regeneration was obtained without an intermediate subculture. This is a new, interesting morphogenetic pathway for the genetic transformation in wheat. In fact, with the regeneration of whole plants by this method, the risk of somaclonal variation often observed in transformation process can be avoided.

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