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Novel In Vivo Regeneration System of Tomato

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ABSTRACT

Plant in vitro regeneration is an important constituent part of horticulture technology and modern molecular breeding. However, many elite commercial cultivars are difficult to culture and regenerate. We need In-depth regeneration mechanism research to address the barrier. Here, we developed a simple, high-efficiency tomato in vivo regeneration method through removing all shoot apex of an intact tomato plant. This indirect regeneration via an intermediate callus phase are not introduced by plant growth regulators. We propose that this process can be used as a model system to investigate the mechanisms that regulate indirect regeneration of higher plants.

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MATERIALS TEXT

The *Solanum lycopersicum* cultivar Micro-Tom was used to induce regeneration in vivo. Determinate tomato Micro-Tom was stored in College of Biological Science and Biotechnology, Shenyang Agricultural University.

ABSTRACT

Plant in vitro regeneration is an important constituent part of horticulture technology and modern molecular breeding. However, many elite commercial cultivars are difficult to culture and regenerate. We need In-depth regeneration mechanism research to address the barrier. Here, we developed a simple, high-efficiency tomato in vivo regeneration method through removing all shoot apex of an intact tomato plant. This indirect regeneration via an intermediate callus phase are not introduced by plant growth regulators. We propose that this process can be used as a model system to investigate the mechanisms that regulate indirect regeneration of higher plants.

BEFORE STARTING

The healthy, strong plants should be used. After resecting primary shoot and all axillary buds, small and weak plants would be difficult to developing callus, even died.
Although flower buds might be observed in tomato with 6-8 leaves, the plant still can be used to induce regeneration in vivo.
The razor blade should be washed and sterilized with 70% ethyl alcohol before cutting.
All axillary buds should be removed. If anyone left, it would hinder the callus formation.
Every three days, loosen the plastic rope and rewire it to make the wounds growth.

1 Tomato plants

Seeds were placed on moistened filter papers for approximately 3 to 4 d until the seeds sprouted in room temperature. The germinated seeds were seeded into a tray of 72 cells filled with a mixture of nutrient soil, matrix, vermiculite and perlite (2:2:1.5:0.5(v/v/v/v)), and grown in a culture room with temperature ranging from 23 to 28 °C and 16/8 h light/dark photoperiod.

2 Excising the main shoot apex

When the seedlings had grown 6 to 8 true leaves, the primary shoot was decapitated horizontally with razor blade. The wound was wrapped to prevent drying.

3 Excising axillary buds

In tomato, a primary shoot shows apical dominance and inhibits outgrowth of axillary buds. After excising the main shoot apex, the dormant axillary buds began to develop immediately to replace the lost shoot apex. Resect all axillary buds that appeared after decapitation.

4 Callus formation and adventitious shoot differentiation

About one week after excising the main shoot apex, callus would be observed on the primary stem wounds first, and then the axillary buds wounds. When the callus entered its differentiation stage, a large number of purple dots appeared on its surface, and finally the shoots appeared to regenerate throughcallus.

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