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The Efficient Regeneration of the Potato (*Solanum tuberosum* L.) cv. Igor in vitro

By

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Key words: Potato, regeneration, zeatin riboside.

Summary

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An efficient protocol for shoot regeneration of potato *Solanum tuberosum* L. cv. Igor was established for use in *Agrobacterium*-mediated transformation. Petioles, internodes, and leaf explants in combination with different plant growth hormones, especially different concentrations of zeatin riboside (ZR), were tested. Shoot regeneration was most successful on callus derived from internode tissue cultured on callus induction medium supplemented with 2.5 mg/l ZR, 0.2 mg/l NAA, 0.02 mg/l GA3 (LDR(3) medium as described by WEBSTER & al. 1994) for two weeks and then transferred to a shoot induction medium with 2.5 mg/l ZR. The regeneration potential of cv. Igor was compared with the potato cv. Désirée.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world. Therefore, many efforts have been made to improve its quality and to introduce different resistances to pathogens that may cause serious damage.

The transformation of potato plants by *Agrobacterium tumefaciens* has become a routine method for introducing genes into some varieties (DALE & HAMPSON 1995, EDWARDS & al. 1991, PERL & al. 1993). But there are great differences among varieties regarding transformation as well as the regeneration process (DALE & HAMPSON 1995).

Cultivation of the Slovenian potato cultivar Igor has been seriously affected by infection with PVY^{NTN}, which causes potato tuber ring necrotic disease.

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Because of the serious damage caused by the disease, cv. Igor practically disappeared from the Slovenian market where it was one of the most important potato cultivars. Therefore our objective is to introduce virus resistance to this particular cultivar by transformation. In this paper we describe an efficient regeneration method, a prerequisite for efficient transformation. The results were compared with the easily regenerating potato cultivar Désirée.

Materials and Methods

The potatoes *Solanum tuberosum* L. cv. Désirée and Igor were grown as node cultures and micropropagated every 4-5 week on MS medium (MURASHIGE & SKOOG 1962) supplemented by 3% sucrose and 0.7% agar. This basic MS medium was used as the basis for callus and shoot induction media.

For the regeneration studies different explants were used: petioles, internodes, and leaf parts. The leaf parts were cultured with the upper surface of the leaf in contact with the MS medium supplemented with 3% sucrose, 0.7% agar, and different hormones. Hormones were filter sterilised.

Callus induction medium with 0.02 mg/l NAA, 0.02 mg/l GA3 and 2.0 mg/l ZR (LDR medium of WEBSTER & al. 1994) was used for cv. Désirée. The experiment was repeated four times in the season.

Explants of cv. Igor were cultured on callus induction medium, supplemented with 2.5 mg/l ZR in combination with 0.2 mg/l NAA, 0.02 mg/l GA3 (LDR(3) medium of WEBSTER & al. 1994) for two weeks. Then the explants were transferred to shoot regeneration medium with 1.0, 1.5, 2.0, or 2.5 mg/l ZR.

In another experiment, explants were cultured directly on the shoot regeneration medium with 3mg/l ZR. Callus induction medium with 3 mg/l ZR in combination with 0.2 mg/l NAA, 0.02 mg/l GA3 was used as a control. Explants of cv. Igor were transferred to fresh media every two weeks. Individual explants were cultured in glass tubes capped with plastic covers.

All regenerated shoots were rooted on MS medium, 3% sucrose, 0.7% agar, without hormones.

Cultures were maintained in growth chambers at 21 ± 1 °C, $50 \mu\text{Mm}^{-2} \text{s}^{-1}$ illumination by Osram L 18W20 lamps and a 16 hours day/8 hours dark photoperiod.

10 petioles, 10 internodes, and 10 leaf explants were used for each medium in one experiment. After 4 weeks for cv. Désirée and 9 weeks for cv. Igor the number of explants forming shoots, the length and the number of shoots were determined. Only shoots longer than 5mm were counted and measured. Student's-t test was used to evaluate statistical differences.

Results and Discussion

The easily regenerating potato cv. Désirée was used as a control for cv. Igor. Shoot regenerated from most explants, only 4% failed to produce shoots. Leaf and internode explants produced more and larger shoots than petioles (Fig.1, data not shown). Even though the experiments were not designed to study the effect of the season on regeneration, we found that the time of the experiment was important for shoot induction. The number of shoots increased from experiments in the beginning of September when the lowest number was detected, to March (Fig. 2). The length of the shoots was about the same in all experiments (Fig. 2).

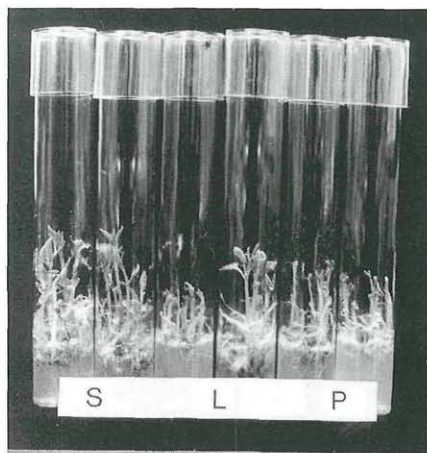


Fig. 1. The regeneration of different explants of potato cv. Désirée after four weeks in culture (S = stem, L = leaf, P = petiole).

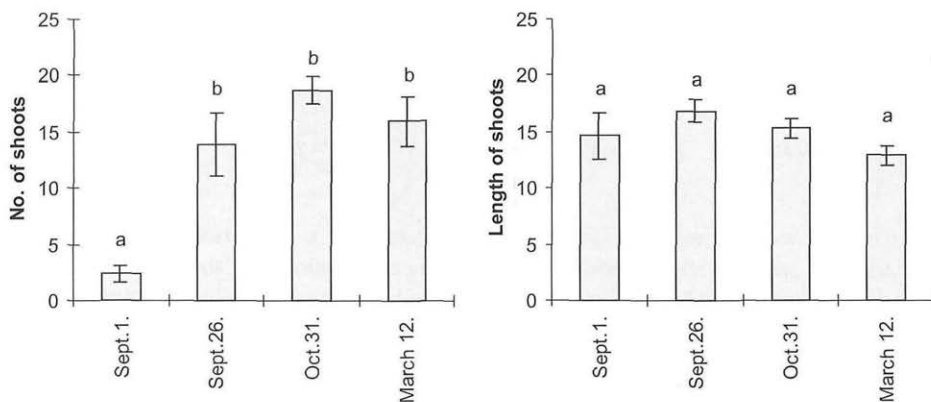


Fig. 2. The number (left) and the length (right) of shoots on leaf explants of potato cv. Désirée after four weeks in culture (average \pm SE). Only shoots longer than 5 mm were counted.

The study of the effect of different explants and hormone treatments on the regeneration of cv. Igor is shown in Fig. 3. The greatest number and length of shoots occurred from internodes at all concentrations of ZR. The number of shoots on internodes and leaves increased with the increasing concentration of ZR. In parallel the length decreased except on leaf explants, where the length increased with increasing concentrations of ZR in the media.

A comparison of two initiation media, the callus induction and shoot induction medium, showed that more and larger shoots were formed if the explants were initially maintained on callus initiation medium (Fig. 4 and Table 1).

Different methods were used to induce shoot regeneration in different potato cultivars (HULME & al.1992). The genotype, explant type, and culture media influence the regeneration potential (CARPUTO & al. 1995). DALE & HAMPSON 1995 found that among 34 potato varieties tested, only half of them regenerated shoots on tuber disks.

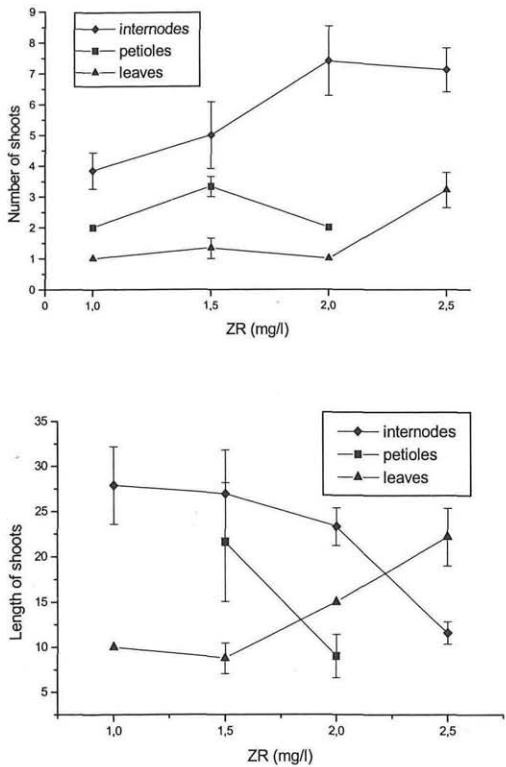


Fig. 3. The influence of different explants and concentrations of ZR on shoot regeneration of *S. tuberosum* cv. Igor. Figure shows the average number (upper) and the length (lower) of shoots per explant \pm standard error. Explants were grown on callus induction medium for 14 days before transfer to shoot induction media.

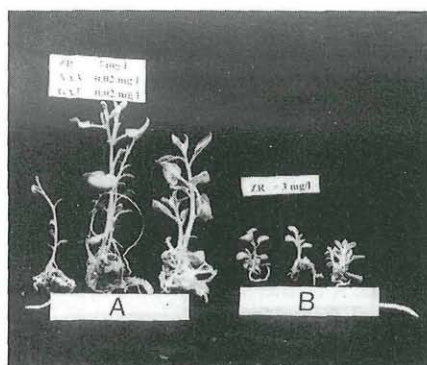


Fig. 4. The comparison of shoot regeneration in cv. Igor from explants first cultured two weeks on callus induction medium (A) or shoot induction medium (B) after nine weeks in culture.

Table 1. The comparison of number and the length of shoots in cv. Igor first cultured two weeks on callus induction medium (0.2 mg/l NAA, 0.02 mg/l GA3, 3 mg/l ZR) or shoot induction medium (3 mg/l ZR) after nine weeks in culture.

	No. of shoots		Length of shoots	
	Internodes	Leaves	Internodes	Leaves
Shoot induction medium	2.9 ± 0.4	1.3 ± 0.3	7.9 ± 0.5	9.5 ± 1.7
Callus induction medium	7.0 ± 1.3	6.5 ± 1.3	21.7 ± 2.4	24.0 ± 3.1

In our experiments a comparison of the potato cv. Igor with cv. Désirée showed that cv. Igor had poorer and slower regeneration ability than cv. Désirée. The first regenerated shoots appeared after two weeks with cv. Désirée, while only after four weeks with cv. Igor. The average number of shoots per explant was also smaller with cv. Igor. The length of shoots with cv. Igor after nine weeks of subcultivation was still smaller than with cv. Désirée after four weeks.

The regeneration procedure described in this paper is suitable for the transformation of potato plants cv. Igor.

Acknowledgements

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