Regeneration of *Solanum tuberosum* L. cv. Tomensa: induction of somatic embryogenesis in liquid culture for the production of "artificial seed"

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Summary

Somatic embryogenesis from excised shoot tip meristems of a tetraploid potato cultivar (*Solanum tuberosum* cv. Tomensa) was studied. Shoot tip meristems were excised from 3 - 4 weeks old *in vitro* propagated plants. Calli were induced in medium supplemented with 3 mg/l NAA and 0.25 mg/l BAP. After cultivation of the resulting calli in multiplication medium supplemented with 3 mg/l NAA and 1 mg/l BAP for 15 – 20 days, the hard green calli were transferred to embryo formation medium supplemented with 0.1 mg/l GA3 and 0.05 mg/l zeatin. Embryo formation was detected after 30 – 35 days. Histological studies confirmed the bipolar nature of the embryos. They developed to normal plants when transferred to soil and grown in a greenhouse.

Zusammenfassung
Regeneration der Kartoffelsorte Tomensa: Induktion somatischer Embryogenese in Flüssigkultur zur Produktion von vegetativen Samen

Die Induktion von somatischen Embryonen aus Sproßspitzenmeristemen einer tetraploiden Kartoffelsorte (*Solanum tuberosum* cv. Tomensa) wurde hier untersucht. Sproßspitzenmeristeme wurden aus 3 - 4 Wochen alten *in vitro* vermehrten Pflanzen herauspräpariert und die Kallusbildung in einem Flüssigmedium durch die Zugabe von 3mg/l NAA und 0,25mg/l BAP induziert. Nach der Kultivierung der daraus resultierenden Kalli in einem Vermehrungsmedium mit 3mg/l NAA und 1mg/l BAP über 15 - 20 Tage wurden die harten grünen Kalli in ein Embryo-Entwicklungsmedium mit 0,1mg/l GA3 und 0,05mg/l Zeatin überführt. Die Embryobildung wurde nach weiteren 30 – 35 Tagen beobachtet. Histologische Schnitte an einem Ultramikrotom bestätigten die bipolare Struktur der Embryonen. Die daraus gebildeten Pflanzen wurden in Erde überführt und entwickelten sich im Gewächshaus zu normal wüchsigen Pflanzen die Knollen bildeten.

Introduction

The potato belongs to the world's most economically important food crops. Vegetative propagation is its major disadvantage for seed potato producers and farmers. Distribution of tissue-borne viruses and bacteria is known as a common problem caused by the asexual method of propagation from seed potatoes in the field. Other related problems for the potato industry are transport and storage of tubers especially in developing countries. An established alternative method for the production of virus-free plants and seed potatoes performed by commercial breeders, is the fast clonal propagation *in vitro* (Espinoza et al. 1986). This method bears the risk of contamination and demands high labour costs and expensive laboratory and culture rooms. In addition the *in vitro* production of plantlets is followed by a greenhouse period with high energy costs. To this day no cost-efficient "low risk" alternative method for the fast and secure propagation of seed potatoes exists. The production of artificial (synthetic) seeds based on somatic embryos could well be a potential solution for seed potato producers and farmers. Redenbaugh (1993) defines synthetic seed as somatic embryos inside a coating where the coating may serve as the synthetic endosperm and is supposed to protect the embryo from mechanical damage. Calcium-Alginate beads are commonly used as a coating. Calcium-Alginate hollow beads show a better performance as protectants against mechanical damage and better rates of emergence (Patel et al. 1997, Patel et al. 2000). Aim of the investigation described in this paper is to develop a reliable procedure to induce somatic embryogenesis in liquid culture as a preliminary stage for the production of potato embryos in a bioreactor. Tomensa was selected as a common cultivar.

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Materials and Methods

Plant material

Shoot tip meristems were dissected from *in vitro* propagated potato (*Solanum tuberosum* cv. Tomensa) microplants. Microplants were grown on MS-medium, Murashige and Skoog (1962), supplemented with 10 g/l sucrose without organic compounds. The medium was solidified with 3.4 g/l gelrite. The pH of all media was adjusted to 5.8 before autoclaving at 1.4 bar for 10 min. Cultivation took place under white light at 3000 Lm/m² with a photoperiod of 12 h at 22° C. After three to four weeks of cultivation shoot tip meristems were excised under a binocular microscope under sterile conditions.

Induction of embryogenic tissue

The excised shoot tip meristems were first cultivated in liquid CI–medium (Table 1) for 25 to 33 days. 15 shoot tip meristems were placed in each 100 ml Erlenmeyer flask filled with 25 ml liquid CI-medium. They were incubated on a shaker with 75 movements per minute under 12 hours of light with an intensity of 850 Lm/m². Temperature was held at 25 ± 1 °C.

The calli were transferred to liquid CM–medium for multiplication under the same environmental conditions. Cultivation time was 15 to 20 days in CM-medium (Table 1). Finally calli were transferred to MSEF-medium (Table 1). When embryos were visible, calli were transferred to solid MS-medium without phytohormones.

Histological methods

Somatic embryos were embedded in LR-White according to Menge-Hartmann and Höppner (1995). The cross sections were prepared with glas knifes on an Ultramicrotom (Om U2, Reichert Austria, Wien) with a strength of 2µm. Sections were stained with toluidine blue solution (0.5 % in water) on the microscopic slides for 10 minutes.

Table1: Composition of cell culture media
Tab.1: Zusammensetzungen der Nährmedien

	Callus induction CI	Callus multiplication CM	Embryo formation MSEF
Major elements	**mg/l**	**mg/l**	**mg/l**
KNO_3	1000	500	1900
NH_4NO_3	-	-	1650
$(NH_4)SO_4$	250	200	-
$CaCl_2 \times 2H_2O$	250	200	440
KH_3PO_4	50	170	170
$MgSO_4 \times 7H_2O$	185	370	370
Na_2EDTA	18.65	37.3	37.3
$FeSO_4 \times 7H_2O$	13.925	27.85	27.85
Minor elements			
H_3BO_3	3.1	6.2	6.2
$MnSO_4 \times 4H_2O$	11.15	22.3	22.3
$ZnSO_4 \times 7H_2O$	4.3	8.6	8.6
KI	0.415	0.83	0.83
$Na_2MoO_4 \times 2H_2O$	0.125	0.25	0.25
$CuSO_4 \times 5H_2O$	0.0125	0.025	0.025
$CoCl_2 \times 6H_2O$	0.0125	0.025	0.025
Carbon source			
Sucrose	20.000	20.000	20000
Glucose	10.000	10.000	-
Growth hormones			
NAA	3	3	
BAP	0.25	1	
GA3	-	-	0.1
Zeatin	-	-	0.05

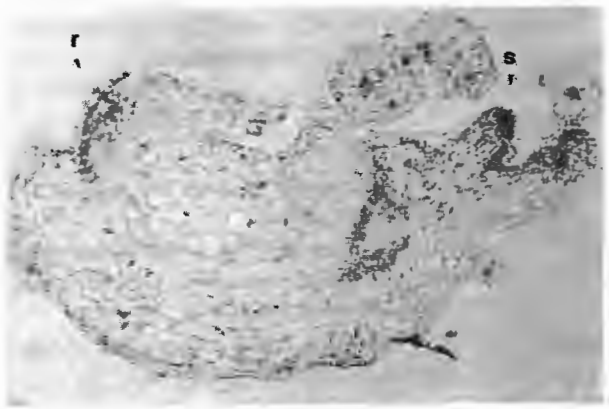

Fig. 1: Cross section of somatic embryo 1cm = 0,22mm
s = shoot, r = root
Abb. 1: Histologischer Schnitt eines somatischen Embryos s = Sproßpol, r = Wurzelpol

Fig. 2: Rooted somatic embryo 1cm = 0,4mm
s = shoot, r = root
Abb. 2: Bewurzelter somatischer Embryo;
s = Sproß; r = Wurzel

Results

In the callus induction medium (CI) 50 % of the shoot tip meristems responded to the phytohormones 1-naphtylacetic acid (3mg/l NAA) and 6-benzylaminopurine (0.25mg/l BAP). In control, almost all shoot tip meristems regenerated on solid medium without hormones.

Vital meristems started to produce hard callus after approximately 20 days of culture in CI-medium. But some of the calli turned brown and failed to proliferate during further cultivation. Approximately 10 % of the shoot tip meristems were still vital after cultivation in CM-medium supplemented with 3 mg/l NAA and 1 mg/l BAP and had produced hard green calli of about 0.5 cm in diameter with brownish parts.

Callus differentiated after 30 to 35 days in MSEF-medium supplemented with 0.1 mg/l gibberellic acid (GA3) and 0.05 mg/l zeatin to embryos up to torpedo stage. Embryos appeared on callus which was very hard and had a fresh dark green colour. Proliferation and production of embryos continued for more than 60 days after calli were transferred into hormon-free MS-medium. At least one piece of callus produced up to 26 embryos. They were only loosely connected to the calli. Histological studies (Fig. 1) revealed that embryos had a clearly bipolar structure.

The isolated embryos germinated on hormon-free MS-medium and developed first shoots and later roots on the same medium (Fig. 2).

Rooted plants (Fig. 3) were transferred to soil and grown in a greenhouse where they showed normal development similar to *in vitro* propagated plants. They produced normal tubers.

The same procedure on solid medium with twice the concentration of the salts in CI-medium resulted in embryos which also grew into vital plants producing tubers from 1 to 4 cm in diameter. Identical results were

Fig. 3: Plants from somatic embryos
1cm = 0,83cm
Abb. 3: aus somatischen Embryonen gebildete Pflänzchen

achieved when zeatin in the MSEF-medium was replaced by the equal concentration of BAP.

Discussion

Somatic embryogenesis from shoot tip meristems of a tetraploid potato cultivar *Solanum tuberosum* cv. Tomensa is reported in this paper for the first time.

There are various reports on somatic embryogenesis in potato anther culture for the production of dihaploid and monohaploid plants (Dunwell and Sunderland, 1973; Sopory, 1977; Johansson, 1986; Shen and Veilleux, 1995). For the production of artificial seeds of tetraploid cultivars the ploidy of the mother plant has to be maintained, therefore anther culture is not practical for the production of seeds. Signs of embryogenic capacity in potato tissues were observed earlier by Lam (1975) and Bragdø Aas (1977). They obtained shoot formation from undifferentiated callus which originated from potato tuber tissue. Tubers that grew in soil bear the risk of virus infection, which makes the method useless for the production of artificial seeds. Somatic embryogenesis from miscellaneous explants were described later: Pretova and Dedicova (1992) studied direct and indirect somatic embryogenesis from unripe zygotic embryos on solid medium. The yield of the direct somatic embryogenesis was too low for mass propagation. Zygotic embryos naturally vary in their genetic uniformity and they are not a suitable base to produce artificial seeds of adequate genetic uniformity. Garcia and Martinez (1995) reported somatic embryogenesis from stem nodal sections on solid medium after more than six month of culture. In our experience it took only 80 days to obtain somatic embryos in liquid culture. The production of callus from shoot tip meristems was first described by Chandra et al. (1985) for a diploid cultivar. They studied growth of callus from shoot tip meristems in liquid culture and regenerated plants from embryoids. No precise description is given of the time frame for embryogenesis. Embryoids developed shoots first and were then made to root on rooting medium containing 0.2 mg/l GA3. Shoot tip meristems are the least infected part of the plant. They are a good base for mass propagation of virus-free plants. The current paper describes a method of producing somatic embryos from shoot tip meristems of a tetraploid cultivar in liquid culture after 80 days which root without a special medium. Optimization of media and environmental conditions will be necessary to prevent the early death of shoot tip meristems and obtain a higher yield of embryos. For the commercial production of artificial seeds scale-up of the somatic embryogenesis in a bioreactor will be necessary to produce large amounts of embryos in a short time which then can be encapsulated in hollow beads.

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