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Plasmolytic behavior of the donor cell may affect pr response

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Protoplast donor tissues (leaves of shoots in culture) fro *num etuberosum*) and two woody species (*Populus a* Crandon and *Betula platyphylla szechuanica*) were comp range of osmotic agents and potentials. Cells from b species proven to be amenable to protoplast division an readily at higher osmotic potentials than cells from *Bet* prolonged culture after protoplast isolation. *Betula* lea persistent membrane-to-wall attachments and many faile extreme osmolarity. Although their leaves exhibited s photosynthetic capacity was lost from *Betula* protoplasts by *Solanum* protoplasts. Differential stress after isolatio vital staining, but only *Solanum* and *Populus* gave bot high plating efficiencies in continued culture.

Key words – *Betula platyphylla szechuanica,* plasmolysis *dentata,* protoplasts, *Solanum etuberosum.*

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Introduction

Although protoplasts have been isolated and cultured from an increasingly wide range of species, plant regeneration has been restricted to a limited number of genera. Chemical and physical microenvironmental factors that govern culture success are still empirically tested to develop protoplast systems, and vital staining is commonly used to assess the integrity of protoplasts after isolation. Although genetic factors are often implicated, specific causes for the poor regenerative capacity of apparently healthy, viable protoplasts can seldom be identified. Tissue-specific reactions to protoplast isolation depend on osmotic, plasmolytic, and other proper-

and protoplast quality. Stress of protoplasts may not be re while subsequent performanc ously impaired (Hampp et al.

This paper compares the characteristics and protoplast cies amenable to protoplast c *sum* and *Populus alba* × *P. gr* and the recalcitrant donor *Bet ica* under standardized shoot c isolation, and protoplast cultu established (Pellow and To McCown 1986, Smith and Mc and plasmolytic behavior of d

tures were generated as previ-
and Towill 1986, Russell and
d McCown 1983) at 27°C under
1 40 μmol m^{-2} s^{-1} light intensity
W cool white fluorescent lamps
Danvers, MA, USA). Cultures
ass jars capped with Magenta B
Chicago, IL, USA) for 30–40
for analysis or protoplast isola-

Leaf sections 80–100 μm thick
to the midrib with an Lancer
PI Inc., St. Louis, MO, USA),
water, then sequentially trans-
gradually increasing concentra-
sucrose, KCl, or a salt solution
rts of a 1 *M* KCl solution and 1
ion, then diluting with distilled
l osmolarity of the plasmolyti-
e common osmotic agents of
tica of smaller molecular size
used to insure penetration
nd solutions containing potas-
tight adherence of the plasma-
Solution osmolarity was deter-
0C Vapor Pressure Osmometer
some treatments, sections were
asmolyticum of low osmolarity
a), equilibrated for 15 min, and
into stepwise increasing osmo-
0 mmol/kg in 100 mmol/kg in-
equilibration period of 10 min
c potential. In parallel observa-
were transferred directly from
rapid plasmolysis. 25 cells sec-
of 15 sections were observed
treatment, to ascertain the oc-
nape of plasmolysis.

culture. Protoplasts were iso-
s of shoots in culture as previ-
and Towill 1986, Russell and
nd McCown 1983, Smith et al.
ssed using fluorescein diacetate
otoplasts were plated at a den-
plasts ml^{-1}. To estimate initial
ts $experiment^{-1}$ were counted,

thick) were used to reduce heat accumulation. Two 150 W spot lamps (General Electric, Columbus, OH, USA) were shielded with neutral density gradients to achieve light intensities of 890 μmol m^{-2} s^{-1}. Leaf tissue slices (about 2 × 5 mm) were submerged in a reaction mixture (3.0 ml total volume) containing either 50 m*M* N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, 0.5 m*M* $CaCl_2$, and 20 m*M* $NaHCO_3$ or protoplast rinse medium (Smith and McCown 1983) and 20 m*M* $NaHCO_3$, at pH 7.5. Protoplasts were assayed only in the latter (osmotically-buffered) solution at a density of 10^6 protoplasts ml^{-1}. The media was purged with air prior to the assay. Respiration was monitored on the same apparatus by occluding all the light with a black cloth. Chlorophyll was extracted and read spectrophotometrically in 96% ethanol and quantified according to Wintermans and DeMots (1965).

Results

Plasmolysis studies. In all tested solutions, *Betula* leaf cells required lower osmotic potentials for plasmolysis, and except in solutions containing KCl, required a much longer incubation period than *Solanum* or *Populus* cells before rounded (convex) plasmolysis of the cell's protoplast was observed (Tab. 1). For *Solanum* cells, attachments causing initially concave (tent-like) plasmolysis (in mannitol or sucrose) were released within 20 min, and rounded plasmolysis was observed in all solutions in

Tab. 1. Plasmolytic behavior of spongy mesophyll cells from *Solanum etuberosum, Populus alba* × *grandidentata,* and *Betula platyphylla szechuanica* in the presence of selected ions. Sections were gradually transferred into solutions of increasing osmolarity. Over 95% of *Solanum* cells and 85% of *Populus* cells plasmolyzed in all solutions. Only 60–80% of the cells from *Betula* sections exhibited plasmolysis; most of these cells in mannitol, $CaCl_2$, and sucrose failed to recover from concave to convex shape even after several hours in plasmolyzing solutions.

Tissue source	Plasmolyticum	Lowest plasmolyzing osmolarity (mmol/kg)	Initial plasmolysis shape	Time required to initiate convex plasmolysis (min)
Solanum	Mannitol	600	concave	20
	$CaCl_2$	500	convex	10
	Sucrose	500	concave	20
	KCl	500	convex	10
	KCl:$CaCl_2$	500	convex	10
Populus	Mannitol	600	concave	30

over 95% of the cells (Tab. 1). For *Populus* cells, over 85% of the cells plasmolyzed, and the initially concave plasmolysis observed in mannitol, $CaCl_2$, or sucrose reverted to convex plasmolysis rapidly within 20–30 min (Tab. 1). In contrast, 20–40% of the *Betula* cells failed to exhibit any plasmolysis following extended incubation in low osmotic potentials. Although a very small proportion (3–5%) of the remaining plasmolyzing leaf cells eventually reverted to the convex shape after extended incubation, the majority retained numerous plasma membrane-to-wall attachment (binding) sites. Particularly strong adherence of the plasma membrane to specific wall attachment sites was noted in $CaCl_2$ for *Betula*.

Rapid plasmolysis resuled in initially concave plasmolysis for cells from all three plants in all solutions excluding the KCl or KCl:$CaCl_2$ solutions. While reversion to convex was usually rapid for *Solanum* (10–20 min) and *Populus* (20–30 min) cells, rounding occurred slowly and erratically for *Betula* in only some of the cells after a 45–60 min lapse, and about 20–40% failed to plasmolyze at all. After extended (16–18 h) incubations in mannitol, some *Betula* protoplasts split into two protoplasms prior to rounding.

Protoplast isolation and culture. Comparative protoplast yields from the three sources were: *S. etuberosum* [ca 1.4×10^7 (g tissue)$^{-1}$], *P. alba* × *P. grandidentata* [ca 1×10^7 (g tissue)$^{-1}$], and *B. p. szechuanica* [ca 1×10^6 (g tissue)$^{-1}$]. Initial protoplast viability, as determined by vital staining, was high for all sources (99%, 90%, and 89% for *Solanum, Populus,* and *Betula,* respectively). Protoplasts from both *Solanum* and *Populus* were subsequently cultured through plant regeneration, whereas *Betula* protoplasts, in contrast, ceased development after early stages of cell division, consistent with earlier results (Smith and McCown 1983).

Comparative photosynthesis and respiration. Photosynthetic rates for leaf tissues were similar from both source plants (Fig. 1). Photosynthetic rates for *Solanum* protoplasts expressed on a chlorophyll basis exceeded rates of *Solanum* leaf tissue slices almost three fold. *Betula* protoplasts, however, consistently failed to demonstrate measurable rates of photosynthesis, although respiration was not inhibited (Fig. 1). Respiratory oxygen uptake rates of both plants were considerably higher for protoplasts than for donor leaf tissue (Fig. 1). *Betula* protoplasts had about 40% less chlorophyll content than *Solanum* protoplasts at the same density (10^6

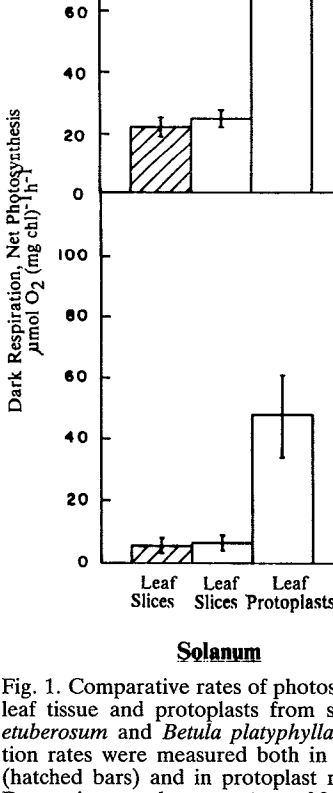

Fig. 1. Comparative rates of photos
leaf tissue and protoplasts from s
etuberosum and *Betula platyphylla*
tion rates were measured both in
(hatched bars) and in protoplast
Data points are the mean ± SE of 3
4 experiments for protoplasts.

failure of a large proportion of
sis even after extended incub
plasmolyzing cells to revert to
dicate that strong binding of th
the cell wall is characteristic of
mann et al. 1985). If detachm
easily in plasmolyzing solutio
likelihood for physiological mea
mechanical damage during cell
isolation; non-uniform contrac
cytoplasmic contents could in
membrane fractures (Lee-Stad
vitt 1972). If a greater degree
cells to incur more damage th
during enzymatic cell wall diges
to the superior culturability

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