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Research article

Evaluation of somatic hybrids of potato with *Solanum stenotomum* after a long-term in vitro conservation

Isabelle Fock [a,*], Cécile Collonnier [b], Danielle Lavergne [b], Sébastien Vaniet [a], Annick Ambroise [b], Jacques Luisetti [a], Hippolyte Kodja [a], Darasinh Sihachakr [b]

[a] *Université de La Réunion, UMR «Peuplements végétaux et bio-agresseurs en milieu tropical», CIRAD-Université de La Réunion, 15, avenue René Cassin, BP 7151, 97715 Saint-Denis Messag Cedex 9, La Réunion, France*

[b] *Université Paris-Sud, Ecologie, Systématique et Evolution, UMR8079, Orsay-CNRS, Bât. 360, Orsay, F-91405, France*

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Abstract

Somatic hybrids of potato with a cultivated relative, *Solanum stenotomum* also called *Solanum tuberosum* Stenotomum group, were evaluated for their physiological and agronomical characteristics as well as the stability of the introgressed resistance to bacterial wilt, caused by *Ralstonia solanacearum*, after a long-term in vitro conservation for more than 5 years. Analysis of photosynthesis showed that the PEPC/Rubisco ratio remained lower than 0.5 for all vitroplants of potato and the somatic hybrids, except for the relative species. This indicates that the carbon metabolism is heterotrophic (ratio > 1) for *S. stenotomum*, and autotrophic for potato and the somatic hybrids (ratio < 1). In both in vitro and greenhouse conditions, potato and the somatic hybrids produced few bigger tubers, while many small tubers were obtained from the relative. The hybrid tubers were morphologically intermediate. The starch content of hybrid tubers was much lower than that of potato, but similar to that of the relative species. Interestingly, the level of bacterial resistance, introgressed from *S. stenotomum* into potato, was shown to be very stable and remained as high as that of the relative after a long-term period of in vitro conservation.

Keywords: PEPC; *Ralstonia solanacearum*; Bacterial resistance; Rubisco; *Solanum tuberosum*; *Solanum tuberosum* Stenotomum group; Somatic hybrids

1. Introduction

Potatoes are grown worldwide and ranked fourth after maize, rice and wheat in the global economy [17]. Because of its economical importance, the cultivated potato is widely studied in order to improve the yield and quality of tubers and increase the resistance to pathogens. So far, much work has been carried out on physiology of tuberization and resistance against diseases, as well as introgression of resistance from wild relatives into cultivated potato [12,23,24,28]. Sources of the genetic diversity used for genetic improvement of potato are from the regions of origin of this crop, i.e. South and Central America [18]. *Solanum stenotomum* Juz. et Buz. is one of the seven cultivated species of potatoes [41] and believed to be the first tuber-bearing species, which had been domesticated around lake Titicaca in Andean high plateau, astride the border between Peru and Bolivia [18]. This diploid species, also called *Solanum tuberosum* Stenotomum group, was thought to be the progenitor of the cultivated potato, *S. tuberosum* [11,18]. Since diploid relatives have various traits of resistance to pathogens, pests or abiotic stress, as well as the ability to adapt to a broad range of climates [17], those plants remain very attractive for potato breeding. Sexual polyploidisation could be involved in the mechanism of compatibility

Abbreviations: DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-2 hydroxyethyl piperazine-*N*′-2 ethanesulfonic acid; MS medium, Murashige and Skoog medium; PAGE, polyacrylamide gel electrophoresis; PEG, polyethylene glycol; PEPC, phosphoenolpyruvate carboxylase; PMSF, phenyl methyl sulfonyl fluoride; PVP, polyvinylpyrrolidone; Rubisco, ribulose-1,5-bisphosphate carboxylase oxygenase; SDS, sodium dodecyl sulphate.

* Corresponding author. Tel.: +262 262 93 81 29; fax: +262 262 93 81 19.
E-mail address: isabelle.fock@univ-reunion.fr (I. Fock).

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between a diploid species and a tetraploid potato. In this case, the ploidy level of the resulted offspring will depend on the endosperm balance number (EBN) of the parents. Thus, diploid *S. stenotomum* is sexually compatible with tetraploid potato provided that the former produced non-reduced gametes [17,18]. However, an intermediate triploid progeny could sporadically be generated by crosses between 2*x S. stenotomum* and 4*x* potato, due to endosperm failure [3] and in this case, backcrosses with *S. tuberosum* L., necessary for bringing back to the tetraploid level, have resulted in only few tetraploid offsprings. Despite the sexual compatibility between *S. tuberosum* Stenotomum group and *S. tuberosum*, the development of tetraploid progeny by using conventional breeding methods is time-consuming. Therefore, somatic hybridization through protoplast fusion offers the alternative possibility to rapidly introduce valuable characters from diploid relative into cultivated potato [10,25,30,33,48].

Somatic fusion is useful for transferring polygenic traits without any detailed identification of the genes, such as the resistance against bacterial wilt caused by *Ralstonia solanacearum*. The bacterial wilt causes heavy damages and is one of the most devastating diseases affecting potatoes worldwide [20]. Since the resistance against this disease is controlled by numerous genes [14,36,39], somatic hybridization by protoplast fusion was found suitable and has successfully been used for the introgression of traits of bacterial resistance from wild relatives into potato. The resulted somatic hybrids, evaluated in vitro, showed different levels of resistance to bacterial wilt [8,9]. However, very little information has so far been available about the physiological and agronomical characteristics and stability of those somatic hybrids.

Therefore, in this work, in view of further exploitation of the somatic hybrids obtained, investigations were extended to study physiological properties of the somatic hybrids, particularly the potential of tuberization and photosynthesis though the activities of PEPC and Rubisco. In fact, in vitro-cultured plants typically showed a low photosynthetic activity, which was considered detrimental to ex vitro acclimatization [7]. Since metabolism changes from heterotrophic to autotrophic status during this critical period, photosynthetic activity will be determinant for the success of acclimatization of in vitro-cultured plants. Likewise, a possible genetic instability of somatic hybrids may occur during in vitro culture, particularly affecting the introgressed bacterial resistance, and so it is necessary to check the stability of the somatic hybrids after several cycles of in vitro multiplication before their further exploitation in breeding programs of potato.

2. Results

2.1. PEPC and Rubisco activities

The PEPC specific activities were significantly lower than Rubisco specific activities, ranging from 4.54 to 12.81 μmol CO_2 mg^{-1} prot h^{-1} and from 7.53 to 42.87 μmol CO_2 mg^{-1} prot h^{-1}, respectively (Table 1). The levels of Rubisco enzymatic activity in *S. tuberosum* were significantly different from those observed in *S. stenotomum* with a very low value. For the somatic hybrids, Rubisco enzymatic activity was significantly higher than that observed for potato. The somatic hybrids also displayed the highest levels of Rubisco enzymatic activities, but the lowest PEPC activities, compared to those of the parents (Table 1). Interestingly, the PEPC/Rubisco ratios in *S. tuberosum* and the somatic hybrids were lower than 0.5, while the ratio in *S. tuberosum* Stenotomum group was estimated at 1.7, indicating that the carbon metabolism is heterotrophic (ratio > 1) for the relative, and autotrophic for potato and the somatic hybrids (ratio < 1).

Western blot analysis of Rubisco revealed the presence of both large and small sub-units in the parental species and somatic hybrids. No significant difference was noticed between the clones tested. In all extracts, a single band of about 100 kDa was detected by PEPC immunoblot analysis.

2.2. In vitro and in vivo tuberization

Under in vitro inducing-tuber conditions, all the vitroplants formed stolons from axillary buds, with visible swellings tips at day 10, and microtubers developed from day 12. The potato and somatic hybrids produced bigger microtubers (74.3–161.0 mg/microtuber), compared to those obtained from *S. tuberosum* Stenotomum group (39.7 mg/microtuber) (Table 2). There was no significant difference between the fresh weight of microtubers of potato and the somatic hybrids harvested at day 24. Similarly, in the greenhouse, *S. stenotomum* produced many small tubers, while few bigger tubers were obtained from potato and the somatic hybrids (Table 3). No significant difference in tuberization was observed between *S. tuberosum* and the somatic hybrids in the greenhouse. Furthermore, as

Table 1
Activities of total soluble proteins and carboxylases (Rubisco and PEPC) examined on 4-week old vitroplants

Genotype	Total soluble proteins per FW (mg g^{-1})	Rubisco specific activity (μmol CO_2 mg^{-1} prot h^{-1})	PEPC specific activity (μmol CO_2 mg^{-1} prot h^{-1})	PEPC/Rubisco ratio
S. tuberosum L.	5.50 b	16.35 c	7.18 bc	0.44 b
S. stenotomum	5.13 b	7.53 d	12.81 a	1.70 a
BS31	6.52 ab	42.87 a	6.92 b	0.16 c
BS44	6.41 ab	31.44 b	4.54 d	0.14 c

Each value is the mean of at least three different measurements in triplicate. Values followed by the same letter within each column are not significantly different at $P = 0.05$.