Information notices issued by ACRE

1. Information notice issued on 22nd December 2025

(Answered on letter below dated '6th Jan 2026')

ACRE have asked the following:

The notice quotes a submission including "*Species from the Morelloid clade have been successfully crossed with S. tuberosum using modern breeding techniques*." This statement has no citation attached in the quoted text and it is unclear which evidence from the review of Jansky 2006 cited later, or from elsewhere, its applicants had in mind. Can the present applicants provide or cite evidence in support of this statement, where 'successfully crossed' refers to one of the traditional processes as defined in subparagraphs 1.6.a.(i)-(vii) of the Precision Breeding Act 2023 (the act), namely:

"(6) In this section "traditional processes" means—
(a) in relation to plants—
    (i) sexual fertilisation,
    (ii) spontaneous mutation,
    (iii) in vitro fertilisation,
    (iv) polyploidy induction,
    (v) embryo rescue,
    (vi) grafting,
    (vii) induced mutagenesis"

As outlined in the act, at Section 1.6.a.viii, somatic hybridisation or cell fusion of plant cells is considered a traditional process where those cells belong to organisms that are capable of exchanging genetic material by a process described in sub-paragraphs 1.6.a.(i)-(vii). Whether genetic material from *Solanum americanum or Solanum nigrum* can be exchanged with *Solanum tuberosum* by one of these processes is currently unclear. A step-by-step description, including any relevant intermediates, of how this exchange could occur between *S. americanum* or *S. nigrum* and *S. tuberosum* via one or more of the processes described at 1.6.a(i)-(vii), would be helpful for informing ACRE's advice.

2. Information notice issued on 22nd January 2026

(Answered under heading 'Multiple strategies for breeding Rpi-amr genes in potato')

ACRE have asked the following:

"...A step-by-step description, including any relevant intermediates, of how this exchange could occur between S.americanum or S.nigrum and S.tuberosum via. one or more of the processes described at 1.6.a(i)-(vii), would be helpful for informing ACRE's advice."

ACRE requests a summary of the notional pathways for inclusion of the proposed cisgenes *Rpi-amr3* and *Rpi-amr1* in the potato gene pool.

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6th Jan 2026

Dear ACRE,

Thank you for your enquiry which highlights a question we had inadvertently left open in our submission.

We would like to add the following citation which addresses your specific question regarding whether *S. nigrum* or *S. americanum* can participate in sexual crosses with *S. tuberosum*.

Eijlander, R., Stiekema, W.J. Biological containment of potato *(Solanum tuberosum)*: outcrossing to the related wild species black nightshade *(Solanum nigrum)* and bittersweet *(Solanum dulcamara)*. *Sexual Plant Reprod* **7**, 29–40 (1994). https://doi.org/10.1007/BF00241885

The abstract reads:
The biological containment of the potato (Solanum tuberosum) was assessed by establishing the crossability of this tuberous crop with the related wild non-tuberous species in The Netherlands, black nightshade (S. nigrum) and bittersweet (S. dulcamara). To circumvent crossability barriers, genotypes with different ploidy number were employed and crosses were performed under different environmental conditions. S. dulcamara was shown to be incongruent with potato at all ploidy levels, while S. nigrum displayed unilateral incompatibility. If S. nigrum was emasculated and used as female, fertilization by potato pollen resulted in berry set and seed development. Emasculation of S. nigrum was essential in this cross, because analysis of the fertilization process demonstrated that this species is highly self-compatible and potato pollen was outcompeted by pollen of S. nigrum. The hybrid seeds derived from this cross did not mature and appeared not to be viable. By application of the technique of embryo rescue of immature embryos, hybrid plants could be obtained. However, these hybrid plants proved to be sterile. These data demonstrate that gene flow by pollen dispersal from potato to its most common wild relatives in Western Europe is highly unlikely. The potato is thus a naturally contained species in this part of the world.

The intention of these authors was to address the question, could transgenes in potato inadvertently transfer to wild *Solanum* species in Europe, such as *S. nigrum* and *S. dulcamara*? They showed that if one uses hexaploid *S. nigrum* as a female (but not male) parent, fertilization can be accomplished with tetraploid *S. tuberosum* pollen. The resulting F1s can give rise to plants via embryo rescue, though these plants were sterile. It was the intention of these authors to make the case that any such hybrids would be sterile. However, this sterility is not surprising since the F1 plants were pentaploid. Due to the specific purpose of their paper, these authors did not test whether using the antimitotic agent colchicine to double the chromosome number of the resulting F1s to decaploid would restore fertility and enable back crossing. Ploidy manipulation is a well-established approach in crop breeding, often allowing for successful overcoming of fertilization barriers and endosperm failure of crosses within the genus *Solanum* (Jansky et al., 2006). It is also worth mentioning that Colon at al. (1992) reported resistance to late blight in the F1s mentioned above. This confirms that the late blight resistance genes such as *Rpi-amr1* and *Rpi-amr3*, which are widespread in *S. americanum* and *S. nigrum* accessions (Lin et al. 2023), can be brought into interspecific crosses with potato via sexual hybridization.

From the standpoint of eligibility of *S. nigrum* (and thus the *S. americanum* gene pool) to be considered cisgenic, these two papers thus indicate that *S. nigrum* is compliant with the below requirements or stipulations of the PBA, specifically:

1. " subparagraphs 1.6.a.(i)-(vii) of the Precision Breeding Act 2023 (the act), namely:
   "(6) In this section "traditional processes" means—
      (a) in relation to plants—
         (i) sexual fertilisation,
         (v) embryo rescue,

We trust this addresses your concerns, and we are happy to address any further questions you may have

Best regards
Jonathan Jones and Agnieszka Witek

Jonathan Jones

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Multiple strategies for breeding *Rpi-amr* genes into potato

Jonathan Jones and Agnieszka Witek

Background

Introgression of genes from wild species into potato by classical breeding is typically a decades-long process. There are often multiple interspecific barriers (Bethke et al., 2017). The challenge is best exemplified by the 50-year timespan between obtaining the first crosses of wild species and the resulting introduction to the market of potato varieties carrying late blight resistance gene *Rpi-blb2* from a wild potato relative *Solanum bulbocastanum* (Fig. 1). In 1959, a cross was made between *S. bulbocastanum* (B, 2x) and *Solanum acaule* (A, 4x) resulting in AB (3x). In 1965, after colchicine treatment to achieve polyploidisation to the hexaploid level, (AB)^2 was crossed with *Solanum phureja* (P, 2x) resulting in (AB)^{2}P (4x). The bridge crosses and chromosome doubling that created (AB)^{2}P enabled the breeders to navigate strong reproductive barriers and cross it with *Solanum tuberosum* (T, 4x). From almost 19,000 pollinated flowers, 36 quadruple ABPT hybrid plants of various ploidy were obtained. These segregated from very susceptible to highly resistant. Through three successive backcrosses to *S. tuberosum*, resistant ABPT (4x) finally yielded the late blight resistant varieties Bionica (2008) and Toluca (2006) (Hermsen, 1994; Haverkort et al., 2016). Other trajectories were easier; hexaploid *Solanum demissum* has been used extensively as a source of *Rpi* genes but most of these *Rpi* genes have been overcome by emerging late blight races (Jo et al., 2014).

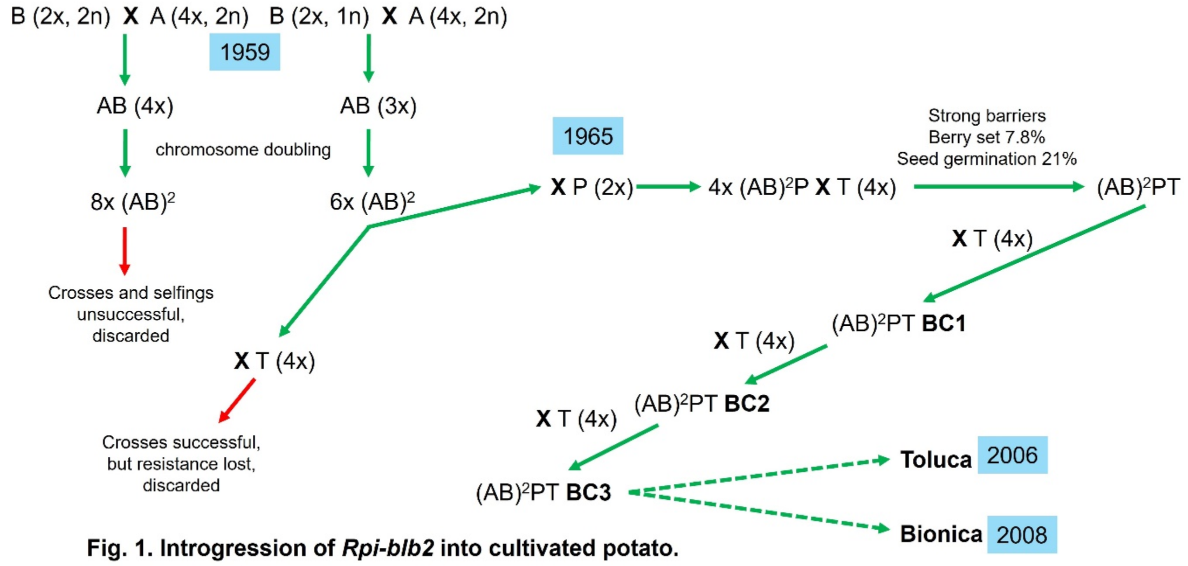

Fig. 1. Introgression of *Rpi-blb2* into cultivated potato.
A, *S. acaule*; B, *S. bulbocastanum*; P, *S. phureja*; T, *S. tuberosum;* **X**, cross; **BC**, backcross

Solanum americanum and its closely related species, *Solanum nigrum* and *Solanum villosum*, are sources of valuable late blight resistance genes (Witek et al., 2016, 2021) and have been long considered promising germplasm to enrich the potato breeding pool. Some initial efforts were made but considering the past breeding experiences and the fast development of modern biotechnology, it seemed quicker to use the alternative approach of gene isolation and plant transformation. Nevertheless, we can envision several breeding strategies that would likely result in the desired introgression of *Rpi-amr* genes into potato if one were prepared to wait 15+ years for them to be bred into productive potato varieties.

Strategy 1

In a previous response, we cited the work of Eiljander and Stiekema (1994) who crossed *S. nigrum* (6x) with *S. tuberosum* (4x). The resulting (5x) sexual hybrid, obtained using *S. nigrum* as the female parent with the aid of embryo rescue, was sterile. For breeders to use this material for *Rpi-amr1* and *Rpi-amr3* introgression into potato, we would undertake the following courses of action to (eventually) enable *Rpi-amr* gene introgression into a useful potato variety.

1. The pentaploid line described above should be chromosome doubled using the mitotic spindle inhibitor colchicine, aiming to obtain a fertile decaploid hybrid in which the chromosomes from each species can pair with their homologs, leaving no unpaired chromosomes at meiosis.

2. Selfed seed of these decaploids should be obtained, and decaploid plants should also be used in back crosses to (2x) and (4x) sect. Petota potato lines. Extensive crosses should be attempted with multiple accessions of a range of bridge species, including diploids (*Solanum verrucosum*, *S. phureja* and others), tetraploids (including *S. acaule*) or hexaploids (e.g., *S. demissum*, previously used as a source of *Rpi* genes in potato breeding). The goal would be to recover fertile hexaploid or octoploid progeny that could be subjected to extensive crosses with a (2x) or (4x) bridge species or (2x) or (4x) potato, with the aim of obtaining a fertile (4x) hybrid that could be crossed and backcrossed to (4x) potato cultivars (Fig. 2).

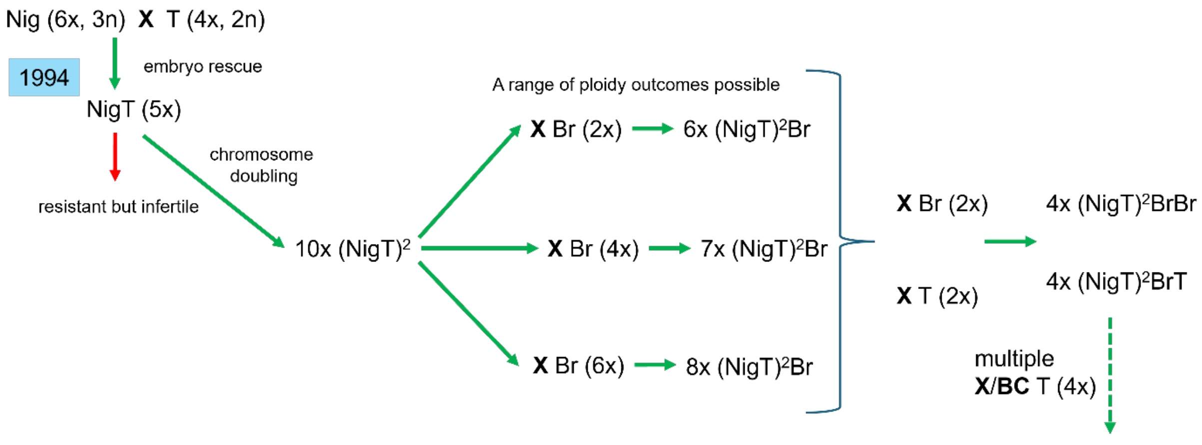

Fig. 2. *Rpi-amr* strategy 1.
Nig, *S. nigrum*; Br, bridge species; T, *S. tuberosum;* **X**, cross; **BC**, backcross

Strategy 2

1. We would use colchicine to achieve a mitotic chromosome duplication of *Solanum americanum* (2x → 4x) and use such a tetraploid line for crosses to bridge species such as *S. acaule*. An *S. americanum* relative *S. villosum* (4x) that carries the alleles of the same *Rpi* genes (Lin et al., 2023) would also be tested in such tetraploid level crosses.

2. The (4x) hybrid plants resulting from crosses with *S. acaule* would be backcrossed several times to *S. acaule*, and the offspring PCR marker-screened for the presence in segregating progeny *Rpi-amr* gene(s) of interest prior to backcrossing to tetraploid potato (Fig. 3).

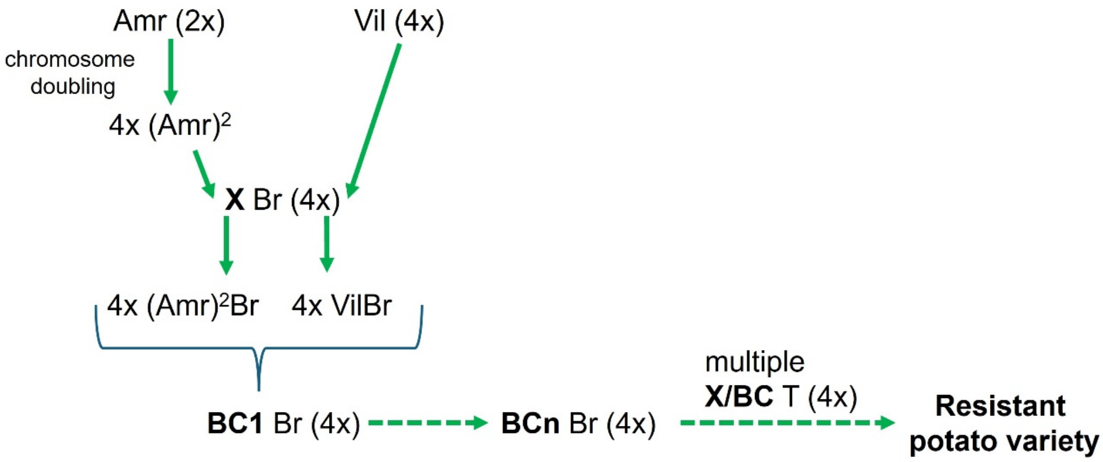

Fig. 3. *Rpi-amr* strategy 2.
Amr, *S. americanum*; Vil, *S. villosum*, Br, bridge species; T, *S. tuberosum;* **X**, cross; **BC**, backcross

Strategy 3

1. An extensive crossing programme would be conducted using *S. americanum* as a male parent in direct crosses to a bridge species like *S. verrucosum* (2x, Endosperm Balance Number (EBN) of 2; Bamberg and Hanneman Jr, 1990) which is known to produce viable seeds when crossed to diploid EBN 1 species (Jansky and Hamernik, 2009; Yermishin et al., 2014).

2. The progeny could be used for further crossing to diploid *S. tuberosum* lines, which, upon chromosome doubling, could be integrated into the gene pool of tetraploid potato cultivars. Alternatively, F1 hybrids between *S. americanum* and *S. verrucosum* would be tested in backcrosses to *S. acaule* (Fig. 4).

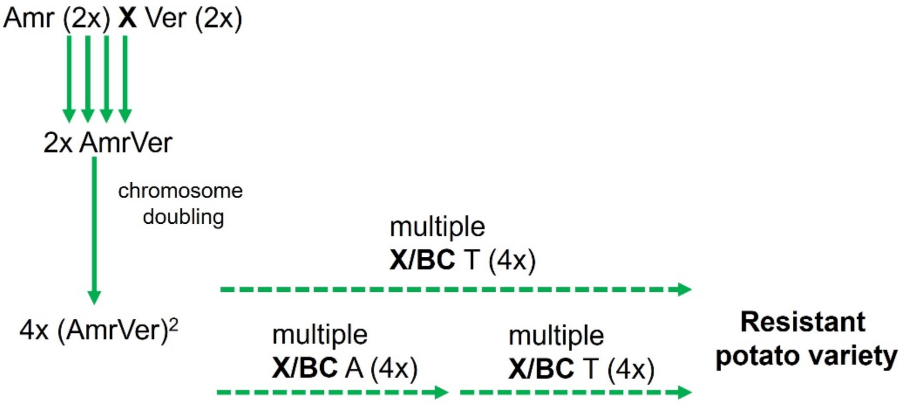

Fig. 4. *Rpi-amr* strategy 3.
A, Bridge species *S. acaule*; Amr, *S. americanum*; Ver, *S. verrucosum*; T, *S. tuberosum;* **X**, cross; **BC**, backcross

Strategy 4

Crossing of *S. nigrum* (6x) to *S. demissum* (6x) should also be attempted, and the recovered hexaploid progeny could be again subjected to extensive crosses with (2x) bridge species or (2x) potato, with the aim of obtaining a fertile (4x) hybrid that could be crossed and backcrossed to (4x) potato cultivars, if necessary via crosses involving the tetraploid bridge species *S. acaule* (Fig. 5).

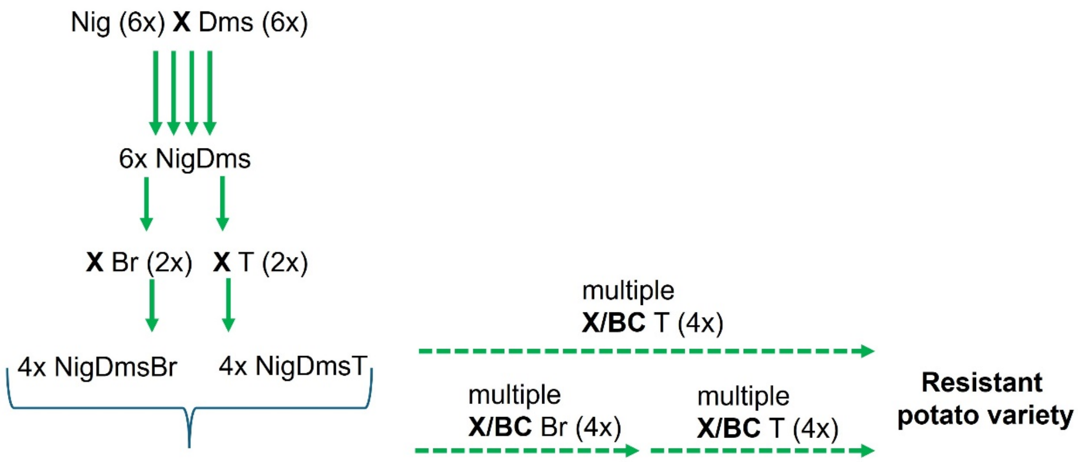

Fig. 5. *Rpi-amr* strategy 4.
Nig, *S. nigrum*; Dms, *S. demissum*; T, *S. tuberosum;* Br, bridge species; **X**, cross; **BC**, backcross

Strategy 5

The Precision Breeding Act states that an organism can be designated as precision bred only if its features resulting from the application of modern biotechnology could have also emerged from traditional processes. The PBA lists the traditional processes as follows:

"(a)in relation to plants—

(i)sexual fertilisation,

(ii)spontaneous mutation,

(iii)in vitro fertilisation,

(iv)polyploidy induction,

(v) embryo rescue,

(vi) grafting,

(vii) induced mutagenesis, or

(viii)somatic hybridisation or cell fusion of plant cells of organisms which are capable of exchanging genetic material by a process within sub-paragraphs (i) to (vii)"

Somatic hybridisation is a valuable tool for introgression of traits from crop relatives, and *Solanum* species are well suited to it (Rakosy-Tican et al., 2026). This approach was used to recruit the *S. bulbocastanum Rpi-blb1 (aka RB)* late blight resistance gene into the *S. tuberosum* breeding pool. Helgeson and colleagues made hexaploid somatic hybrids between *S. bulbocastanum* and *S. tuberosum* and RB-specified resistance was passed on undiminished to progeny of the first and second backcrosses to potato (Helgeson et al., 1998; Douches et al., 1997). BC2 and BC3 backcrosses segregated for resistance to *P. infestans,* and the RB gene was mapped to chromosome 8 (Naess et al., 2000). Lines carrying RB have been incorporated into the potato breeding pool.

S. nigrum can exchange genetic material with *S. tuberosum* by sexual hybridisation followed by embryo rescue (Colon et al., 1992; Eiljander and Stiekiema, 1994); therefore, we consider somatic hybridisation followed by classical genetic backcrossing a valid traditional process and valid approach to breeding *Rpi-amr* genes into potato.

Somatic hybrids between *S. nigrum* and diploid or tetraploid potato were previously reported (e.g., Binding et al., 1982; Horsman et al., 1997; Szczerbakowa et al., 2003). Moreover, first and second backcrosses of *S. nigrum* somatic hybrids with *S. tuberosum* to tetraploid potato were successfully achieved (Horsman et al., 1999); the same report also demonstrated effective incorporation of the resistance-conferring genetic material into the recombining genetic pool of the BC2 progeny and genetic segregation of that resistance. Furthermore, successful somatic fusion experiments were performed between the above mentioned BC1 and BC2 and several potato cultivars (Horsman et al., 2001). Such somatic hybrids would be a good starting point for further extensive backcrosses to potato cultivars, with the aim of retaining resistance, improving fertility and refining the phenotype (Strategy 5a, Fig. 6).

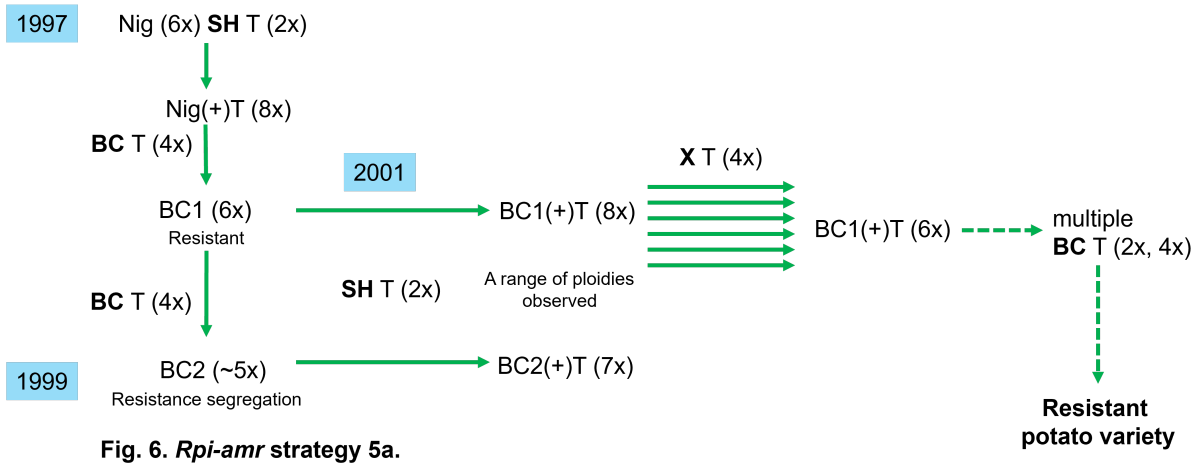

Fig. 6. *Rpi-amr* strategy 5a.
Nig, *S. nigrum*; T, *S. tuberosum;* **SH**, somatic hybridization; **X**, cross; **BC**, backcross

Alternatively, as reported by Helgeson and colleagues for introgression of RB from *S. bulbocastanum* into potato, a hexaploid somatic hybrid between *S. americanum* and tetraploid potato could be created by somatic fusion, and then recurrently backcrossed to tetraploid potato, selecting for progeny that retain the gene of interest (Strategy 5b, Fig. 7). New methods are becoming available to enhance the recovery of such somatic hybrids – grafting followed by selection can also be used (Fuentes et al., 2014). This approach was attempted by Horsman et al (1997) but with only one diploid *S. americanum* accession.

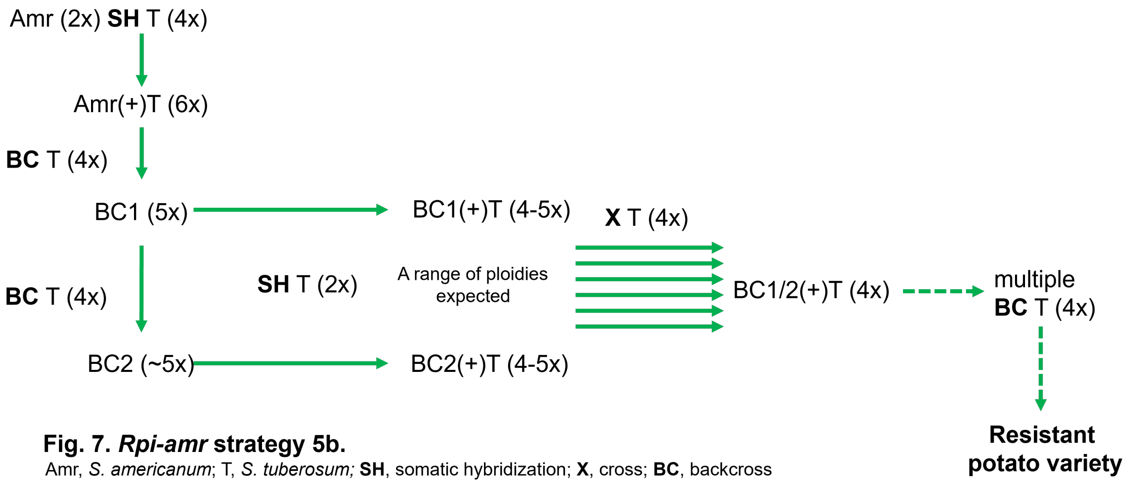

Fig. 7. *Rpi-amr* strategy 5b.
Amr, *S. americanum*; T, *S. tuberosum;* **SH**, somatic hybridization; **X**, cross; **BC**, backcross

In all these strategies, the retention of resistance can now be closely monitored with PCR molecular markers to track *Rpi-amr1* and/or *Rpi-amr3* in segregating progeny, which would accelerate the elimination of plants lacking the gene of interest. This does not change the fact that the breeding process would be long and laborious, and roadblocks might be expected that would require the breeder to course-correct the next steps of the breeding program. Offspring might display a range of ploidy and fertility levels, and resistance segregation. Based on the ABPT hybrids described above and other examples (notably, a quadruple hybrid bearing potato leafroll virus resistance introgressed from *Solanum etuberosum* (Hermsen, 1994), and others), the introgression process might require applying any of the following: choosing the right direction of the crosses (i.e., choice of which line to use as male or female parent) to remedy a unilateral incompatibility; using mentor pollen or double pollination; introducing one or more bridge species to facilitate genetic material transfer (Jansky, 2006); ploidy manipulation by applying chromosome doubling to mitigate hybrid sterility (Hermsen, 1994), using 2*n* gametes or haploidization, or extensive crossing with or without embryo rescue to adjust EBNs (Carputo et al., 1997), exploiting triploid intermediates followed by chromosome doubling (Adiwilaga et al., 1991); somatic hybridisation with (2x) or (4x) potato to improve the fertility of the hybrids (Horsman et al., 2001); and more. A severe bottleneck is usually observed at the first generation of crosses to potato cultivars, which can be overcome by large-scale crosses involving different male and female genotypes, adjusting EBNs and by using ovule cultures or embryo rescue techniques (Hermsen, 1994).

Taken together, with sufficient time, applying a range of such plant breeding techniques and scaling up the process would enable introgression of *Rpi-amr* genes into potato breeding lines.

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