Overcoming interspecific reproductive barriers (IRB) by embryo rescue to capture new sources of resistance to late blight

Abstract
High levels of resistance have been observed in wild potato (Solanum sect. Petota) species endemic to late-blight prone environments of the Peruvian Andes. Six species were selected from the Piurana clade (Fig. 1 and Fig. 2) as sources of putative novel resistance for use in broadening the genetic base of potato. We found that S. cajamarquense, S. chiquitumum, S. pavoceum, and S. piureae were rich in resistance, while S. acroclayum and S. chomphumphum were generally susceptible to infection. Initial interclade crosses with cultivated diploid potato did not yield any seed, despite the wild species genotypes producing plenty of viable pollen. Growth of pollen tubes in the styles of the flowers was generally poor, with only a few flowers setting forming the crosses. Cumulative studies suggest bilateral incompatibility, since crossing of cultivated potato with all species except S. chomphumphum are equally problematic in both directions (Table 1). Pollen tube growth was monitored to identify combinations in which embryos were formed. In vitro methods enabled the culture of 691 embryos from the torpedo and heart stages, and over 480 hybrids were brought to maturity for resistance assessment and fertility studies.

Methodology

Pollen tube growth
Emasculated flowers were pollinated by dipping pistils into gelatin capsules containing pollen. Complete pistils were excised 48 H after pollination and kept in aniline blue (Schreiter & Tiemann, 1977), and kept at 4 °C, for up to one week. Three pistils per cross were boiled in water until soft and placed on a slide in aqueous glycerol, covered with a cover slip and gently squashed. Pollen tube growth was evaluated using an 18 point scale comprised of a matrix indicating the relative amount of pollen tubes observed and the distance they grew in the style in the allotted time according to Trogitz (1991). Fig. 3

Results

Pre- and post zygotic reproductive barriers
Seed set in successful crosses was correlated with the scores of pollen tube growth suggesting that style incompatibilities are the major source of limited seed set. (Table 1). The only species not presenting this IRB was S. chomphumphum of which a single genotype was found to cross readily with cultivated potato as either a pollen or a pistilate parent. 691 hybrids grew normally and over 480 have been readied to late blight. 711 additional embryos failed to develop apical shoots despite nearly normal root development, illustrating post zygotic barriers.

Late blight exposure
The new hybrids were propagated in vitro and established in an Alpha design in each of two field locations (Ocong and Moyobamba, Peru) for natural infection. Whole plants were also inoculated with a zoospore suspension of the complex isolate POX-067 of P. infestans in a greenhouse equipped with humidifiers. AUDPC was calculated from weekly readings of % foliage infected.

Embryo culture
Fruits were harvested 19-27 days after pollination and immature embryos were excised under a stereoscopic dissecting microscope and cultured on Murashige and Skoog basal medium with 1g/l of activated charcoal adjusted to pH 5.6. The embryos were incubated at 18-22°C, with 16 hours light (3000 lux). Excision and classification were based on Spack’s (1985). Fig. 4

Late blight resistance (greenhouse)
A range of reactions from completely susceptible to highly resistant was observed among genotypes of the same cross. Notably, genotypes with AUDPC values lower than the most resistant control (LBR_40) were identified in hybrids from the crosses S. phureja × S. pavoceum [phu × pco], S. stenotomum × S. cajamarquense [stn × cjm] and S. phureja × S. piureae [phu × pur]. Fig. 5

Embryo culture (Table 1)

Table 1: Results of crossing new sources of late blight resistance as males with 2x hybrids, hoplids and diploid native cultivars

<table>
<thead>
<tr>
<th>Cross type: Dizygotes × 2RPM</th>
<th>No. forerunners</th>
<th>No. matres</th>
<th>Genotypes (score 5-8)</th>
<th>Average seeds</th>
<th>Fruit</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>2x Hybrid × phu</td>
<td>12</td>
<td>15</td>
<td>10</td>
<td>25.50</td>
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<tr>
<td>2x Hybrid × pco</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>10.20</td>
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<tr>
<td>2x Hybrid × cjm</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>10.50</td>
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<tr>
<td>2x Hybrid × cjm</td>
<td>6</td>
<td>8</td>
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<tr>
<td>2x Hybrid × stn</td>
<td>10</td>
<td>12</td>
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<td>2x Hybrid × stn</td>
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Reproductive characteristics of the hybrids
Pollinability was determined by staining in aceto-carmine glyceral jelly. Two samples of 3-5 flowers were collected from each genotype and stained for 24 hours. Percentage stainable pollen and frequency of 2n pollen were recorded on samples of 200 pollen grains.

Future perspectives
Despite both pre-zygotic and post-zygotic reproductive barriers, several hundred hybrids have been obtained with 4 species of the Piurana clade. The further characterization of useful traits in the resulting hybrid germplasm, and of interspecific reproductive barriers in Solanum, with new genetic and genomic tools will guide us to more effective use of these wild species in breeding.

To constitute and upgrade a base population
To characterize diversity of S alleles, responsible for incompatibility, in diploid cultivated potato and selected wild species
To introgress novel resistance into advanced populations for collaborative breeding with partner programs

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References