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Adventitious shoot regeneration from *in vitro* leaves of *Aronia mitschurinii* and cotyledons of closely related Pyrinae taxa

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ABSTRACT

The objective of this study was to develop an *in vitro* shoot regeneration procedure and to evaluate the frequency of adventitious shoot regeneration from: (1) *in vitro* leaves of a commercial cultivar of *Aronia mitschurinii* on various media treatments; (2) cotyledons of closely related Pyrinae taxa; and (3) 21 wild *Aronia* genotypes. Optimum regeneration of leaf explants occurred when they were wounded with two transverse cuts along the midrib and placed on Murashige and Skoog (MS) basal media containing 5 µM indole-3-butyric acid (IBA) and 10 µM thidiazuron (TDZ). TDZ was more effective than 6-benzylaminopurine (BAP) as a cytokinin, and IBA was more effective than the no auxin control, 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA). Regeneration from cotyledons of seven Pyrinae taxa was evaluated using 10 µM BAP in combination with 0.1, 1 and 5 µM NAA. Adventitious shoot formation for *A. melanocarpa* and *P. communis* responded best to 1 µM NAA, whereas all other taxa formed a greater number of adventitious shoots on 5 µM NAA. *A. mitschurinii* cotyledon explants produced a significantly greater number of shoots compared with *in vitro* leaf explants. The number of shoots forming per cotyledon explant and the percent of explants forming shoots were both significantly different among the 21 *Aronia* genotypes. Significant differences were observed between the six *Aronia* taxonomic groups for the number of shoots forming per explant. Diploid and tetraploid *Aronia* genotypes produced a significantly greater number of shoots per explant than did triploid genotypes. Regenerated shoots were rooted *in vitro* and plants grew normally in the greenhouse. These results will be useful for future studies using leaf and cotyledon explants for genetic transformation, genome editing and mutation breeding with *Aronia* and related taxa.

1. Introduction

Plants in the Rosaceae family, subtribe Pyrinae, include a number of economically important fruit crops that are beneficial for human nutrition (Hummer and Janick, 2009). Common pome fruits include *Malus* Mill. (apple), *Pyrus* L. (pear) and *Cydonia* Mill. (quince) along with less commonly known fruits including *Sorbus* L. (mountain ash), *Aronia* Med. (chokeberry), *Amelanchier* Med. (serviceberry), *Crataegus* L. (hawthorn), and several other woody plants (Campbell et al., 2007). Interest in aronia fruit has increased because of their high levels of antioxidants and polyphenols (Zheng and Wang, 2003; Wu et al., 2004; Brand et al., 2017) and wide adaptability to various geographic regions with few disease and pest issues (Dirr, 2009; McKay, 2001). However, aronia fruit production uses germplasm that possesses very little genetic diversity (Persson Hovmalm et al., 2004; Leonard et al., 2013; Connolly, 2014) and to protect fruit growers from adverse biological and economical impacts, it will be essential to create novel types of *Aronia* fruits to reduce monoculture.

Traditional breeding approaches typically require long regeneration periods and significant resources for hybridization and selections to introgress genes for desirable traits. Genetic transformation provides an opportunity to transfer desirable target genes and generate unique breeding materials, and this method of breeding has become important, especially for woody fruit species (Gambino and Gribaudo, 2012). Shoot organogenesis from morphogenic explants, including leaf explants and cotyledons have been used for genetic transformation in pear (Gao et al., 2002; Kaneyoshi et al., 2001), apple (Holefors et al., 1998) almond (Miguel and Oliveira, 1999), and apricot (Petri et al., 2008). With recent advances in genetic transformation and genome editing technologies, sufficient protocols for shoot regeneration are necessary for utilization of these methods for the improvement of these economically important fruit crops.

In vitro shoot organogenesis has been reported from *Sorbus aucuparia* leaf and stem explants (Lall et al., 2006), pear cotyledons (Kaneyoshi et al., 2001; Nakajima et al., 2012) and pear leaves (Hennayake et al., 2003; Tang et al., 2008; Bell et al., 2012). There have

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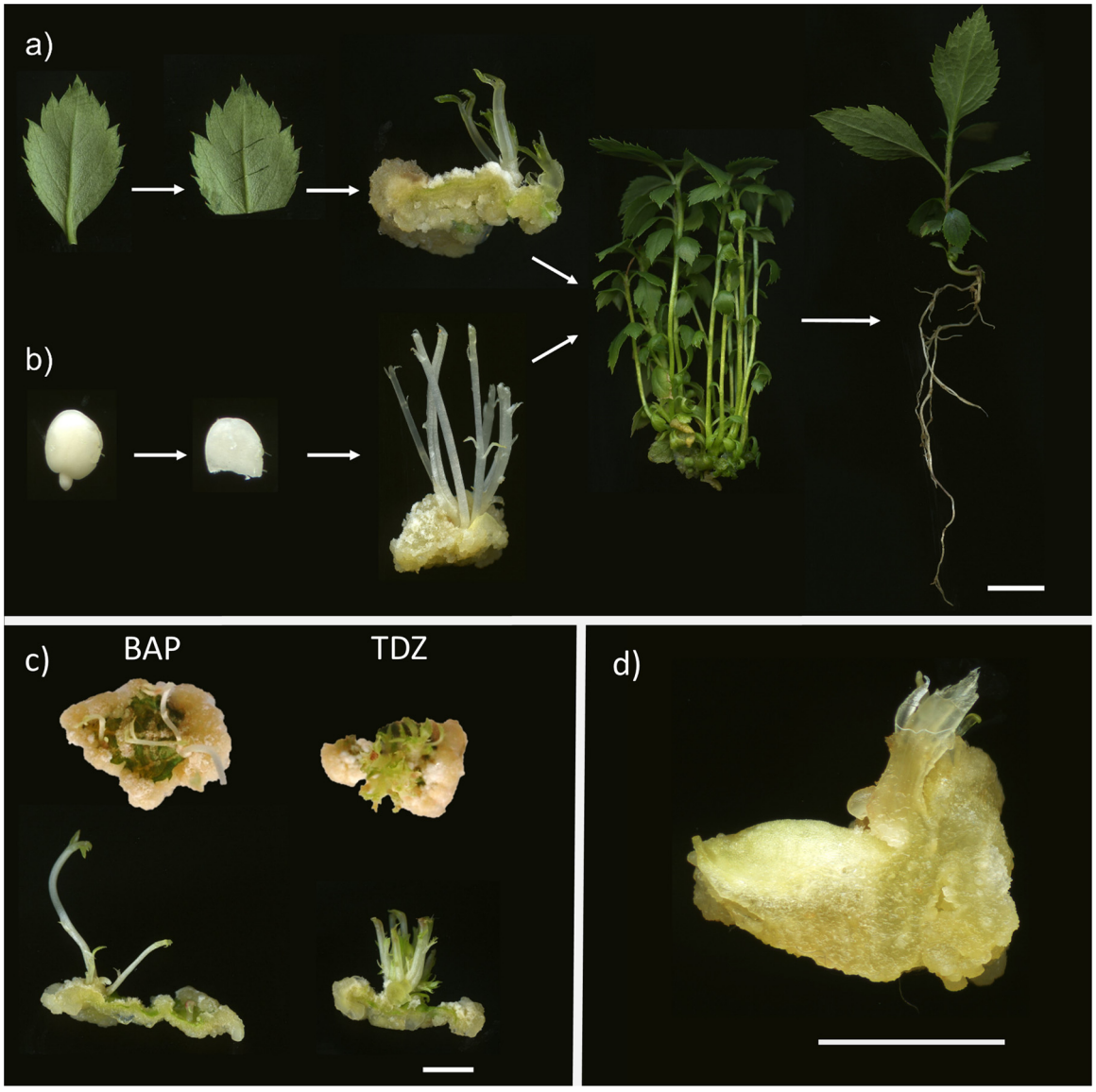

Fig. 1. Explant preparation and process for *Aronia mitschurinii* shoot organogenesis of (a) leaf explant (Experiment I) and (b) cotyledon (Experiment II & III) (*bar 5* mm). (c) Leaf explants after 2 months of culture on media containing 5 μM NAA with either 10 μM BAP or 10 μM TDZ (*bar 5* mm). (e) Cotyledon explants after 5 wk of culture beginning to form adventitious shoots from dedifferentiated callus tissue (*bar 2* mm).

Table 1
Seven Pyrinae accessions used in experiment II.

Species	Parentage	Ploidy	Accession/cultivar	Germplasm source	Germplasm origin
Aronia melanocarpa	–	2*x*	PI 613016	USDA, Ames, IA	Massachusetts
Aronia mitschurinii	×*S. fallax* × *A. melanocarpa*	4*x*	Viking	University of Connecticut, Storrs, CT	Cultivated origin
×*Sorbaronia fallax*	*S. aucuparia* × *A. melanocarpa*	2*x*	None	University of Connecticut, Storrs, CT	Massachusetts
Sorbus aucuparia	–	2*x*	None	University of Connecticut, Storrs, CT	England
×*Sorbaronia dippelii*	*S. aria* × *A. melanocarpa*	2*x*	759-78	Arnold Arboretum, Boston, MA	Germany
Sorbus aria	–	2*x*	None	Sheffield's Seed Co., Locke, NY	Unknown
Pyrus communis	–	2*x*	Bartlett	Sheffield's Seed Co., Locke, NY	Unknown

been no reports for adventitious shoot regeneration from leaves and cotyledons of *Aronia* or cotyledons of *Sorbus*. One objective of this study was to evaluate the frequency of adventitious shoot regeneration from leaves of a commercial cultivar of *Aronia*. A second objective was to examine shoot organogenesis on cotyledons of closely related Pyrinae taxa on media containing various ratios of auxin and cytokinin. The third objective was to examine the regeneration frequency from cotyledons of 21 *Aronia* genotypes comprising six taxonomic groups. The development of improved and more efficient *in vitro* culture protocols for regeneration will be a valuable asset for continued efforts in evaluating and breeding novel genotypes through hybridization, genetic transformation and gene editing.