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Genetically-controlled leaf traits in two chemotypes of *Salix sachalinensis* Fr. Schm (Salicaceae)

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Abstract

Salix sachalinensis has two chemotypes: one biosynthesises ampelopsin as a major component of low molecular weight phenolics in their leaves (A-type), and the other biosynthesises β-D-glucopyranose-1-*trans*-*p*-coumarate (PG1) and β-D-glucopyranose-1-*trans*-cinnamate (PG2) in addition to ampelopsin (AP-type). We investigated phenotypic and genetic variations and clonal repeatabilities of the pubescence density, leaf mass per area (LMA), and concentrations of total phenolics, condensed tannin, ampelopsin, PG1 and PG2. Leaves of wild A-type trees contained significantly higher concentrations of total phenolics and ampelopsin, and lower concentration of condensed tannin than those of wild AP-type trees. In the greenhouse experiment that compared leaf traits between cloned trees obtained from wild chemotypes, there were significant between-type variations in the leaf phenolic concentrations, pubescence density, and LMA. Since chemotypes of cloned trees in the greenhouse were the same as those of wild parent trees, chemotype can be considered as a genetically controlled property. There were also significant within-chemotype variations in the pubescence density, LMA, total phenolics, ampelopsin, PG1, and PG2 concentrations, but not in concentration of condensed tannin for either chemotypes. Genetic variation of leaf traits except for LMA in AP-type was significant. PG1 and

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PG2 exhibited the highest clonal repeatabilities (0.73 and 0.78, respectively). Thus, the ability to produce and the amount of production of PG1 and PG2 are genetically controlled.

Keywords: *Salix sachalinensis*; Chemotype; Ampelopsin; β-D-Glucopyranose-1-*trans*-cinnamate; β-D-Glucopyranose-1-*trans*-*p*-coumarate; Genetic variation; Phenotypic variation; Clonal repeatability

1. Introduction

Secondary metabolites (e.g. phenolics and alkaloids) and pubescence are well-known deterrents and/or attractants of herbivores and pathogens, and can affect host use patterns (Bernays and Chapman, 1994; Schoonhoven et al., 1998). Intraspecific variation in leaf traits of wild plants is well documented, and researchers have been investigating the mechanisms that maintain such variation (Kennedy and Barbour, 1992). To elucidate the maintenance mechanism of intraspecific variation, it is necessary to investigate the relative contribution of genetic and non-genetic variation to phenotypic variation observed in nature (Simms and Rausher, 1992).

Salix sachalinensis is distributed from Japan to Russian Far East (Satake et al., 1989), and is a common willow in Hokkaido, northern Japan. It occurs along riversides and in mesic lowlands (Niiyama, 1990). In general, willow leaves contain phenolics as major secondary metabolites (Palo, 1984). *S. sachalinensis* has two distinct chemotypes (A-type and AP-type) that vary in the profile of low molecular phenolics in the leaves (Mizuno et al., 1989). The A-type produces ampelopsin that varies from 5.6 to 71.2 mg/g d.w. among A-type trees in Sapporo, Japan (Matsumoto and Tahara, 2001). The AP-type produces ampelopsin and two phenylpropanoid glycosides: β-D-glucopyranose-1-*trans*-*p*-coumarate (PG1) and β-D-glucopyranose-1-*trans*-cinnamate (PG2). In addition to these phenolic traits, the undersurface of *S. sachalinensis* leaves is pubescent (Satake et al., 1989). In spite of the existence of continuous (within-chemotype) and discontinuous (between-chemotypes) phenotypic variation, genetic control of this variation in leaf traits has never been examined.

Since willows can reproduce clonally from branch fragments (Newsholme, 1992), it is easy to make clones of parent trees by rooting replicate cuttings. Using this attribute, one can estimate genetic variation of particular traits by cultivating the clones in a uniform environment (Simms and Rausher, 1992). The objective of this study is to assess the phenotypic and genetic variations in leaf traits, both between and within chemotypes, and to investigate how much this intraspecific variation is genetically controlled. Clonal studies provide clonal repeatabilities, which is equal to upper-bound estimates of broad-sense heritabilities.