Reprinted from: Issues in new crops and new uses. 2007. J. Janick
and A. Whipkey (eds.). ASHS Press, Alexandria, VA.

Characterization and Evaluation of Two Chilean Populations of *Geum quellyon*

Susana Fischer, Luis Inostroza, Rosemarie Wilckens, Marisol Berti, Marcelo Baeza, and Edgar Pastene

The Chilean Mediterranean area is a plant biodiversity hot spot with special characteristics due to genetic isolation derived from its geographical limits, the Atacama desert, Andean mountains, Pacific Ocean, polar ice region, and broad climatic zones. Chilean plant species are numerous and 44% of them are endemic (Vogel 2000), and therefore they constitute a unique and exclusive genetic heritage in the world. Many Chilean endemic plants were a main component of indigenous and popular medicine that are still constantly in demand. Human intervention and indiscriminate cultivation of endemic and native species represent a constant threat for its diversity and ecological balance (FIA 2001).

Among Chilean endemic species, the clove herb (*Geum quellyon* Sweet, Rosaceae) is distributed between 32°55' S, 70°40' W and 41°23' S, 71°34' W. *Geum quellyon* is a herbaceous plant, 6 to 100 cm tall, with compound leaves of 3 to 10 pairs of oval leaflets, flowers are in unifloral axes or cymes, each flower has 5 to 8 red or orange petals. The plant has a thick-dark-brown tap root with a sweet clove smell when broken (Fig. 1).

For many years clove herb root has been used by Mapuche Amerindians, and is used in traditional medicine principally as an aphrodisiac. It is also used as tonic, for benign prostate hyperplasia treatment, female climacteric abnormalities (regulator), diuretic, for tooth neuralgia, and human and animal hypertension problems (Muñoz et al. 2001; Hoffmann et al. 2003). However, there are no clinical studies to justify its medicinal use for these mentioned purposes (Muñoz et al. 2001). Antioxidant properties have been demonstrated *in vitro*, but not *in vivo* (Russo et al. 2005).

Therapeutic properties have been described for other *Geum* species. For example, in Sweden popular medicine uses *G. rivale* L. and *G. urbanum* L. to treat inflammatory diseases and dermal wound disinfection (Tunon et al. 1995). British Columbian popular medicine uses antifungal properties of root extracts of *G. macrophyllum* Willd. (McCutcheon et al. 1994).

Clove herb root contains numerous chemical compounds, including hydrolysable tannins, free galic acid and its glycosylated derivatives, ellagic acid, and flavonoids. Hydrocarbons, aromatic compounds, sugars, phytosterols, saturated and unsaturated fatty acids, acylglycerols, and a series of inorganic compounds are also found (Montes s/f; Plantas medicinales y medicina tradicional. s/f; Muñoz et al. 2001; Araneda 2003).

The genus *Geum* present in a complex polyploidy, generating many species groups in different evolutionary stages (Gajewski 1959), and is difficult to classify. To solve this taxonomic problem, Smedmark et al. (2003) developed the first *Geum* phylogenetic study using molecular evidence. The origin of seven polyploid species was determined based on a nuclear phylogenetic gene (GBSSI-gen; Granule-Bound Starch Synthase).

Fig. 1. View of *Geum quellyon* plant and roots.

In Chile, there is a lack of information about *G. quellyon*. The objective of this work was to study seed germination, phenology, and chemical and molecular characterization in two populations derived from different altitudes.

MATERIALS AND METHODS

Assays and evaluations were performed in two *G. quellyon* populations collected between 36 and 38° 30' S latitude and 71° 00' W longitude in Chile, growing in very different hydro environments. The first population (P1) corresponds to a group of accessions collected above 1200 m above sea level, growing in a humid environment beside rivers and streams. The second population (P2) corresponds to an accession group collected below 200 m, growing in a dry hillside environment.

Germination Assay

During the 2005 season, the effect of temperature on P1 and P2 seed germination was studied. Seeds from each population were disinfected with 20% (w/v) benomyl/water solution and were arranged in Petri dishes containing filter paper moistened with distilled water. Seeds were subjected to three thermal treatments (5, 12, 20°C) and two light treatments (with or without light) in growth chambers. Germinated seed number was recorded daily for a two month period. Maximum germination percentage for each seed population was calculated. Data were subjected to analysis of variance (ANOVA) and treatment means were compared by Tukey test ($\alpha = 0.05$) (SAS Institute 1990).

Seeds were germinated in a temperature-gradient bar adapted from Barbour and Racine (1967) and Fox and Thompson (1971) to determine the minimum, optimum, and maximum temperature for germination. The basic unit of the temperature-gradient apparatus is an aluminum bar insulated on three sides with expanded polystyrene, heated with a hot water bath at one end and cooled at the other with a refrigerator cooling system. The bar was covered with moistened filter paper throughout the experiment to ensure water for the germinating seeds. A temperature bar-gradient from 0 to 35°C was used. Thirty seeds were placed in each of the 37 sections of the bar, divided in six cells, which they were used as replications for the combined analysis, although replications were not randomized because of the design of the bar. Once seeds were in place, the bar was covered with a piece of polyesterene and a clear Plexiglas cover. The experiment was conducted in light with 16-h photoperiod. Two runs were conducted. Regression analysis was performed and the cubic equation was fitted to the data from both runs.

Clove Herb Phenology

An *ex situ* study was established at the Universidad de Concepción Experimental Station in Chillan (36°35' S, 72°04' W; 140 m). All plants were obtained from seeds germinated in previous experiments. Plants were grown in plastic bags with a mixture of 1 soil : greenhouse pot mix (Agromix®). Plants were transplanted to the field when they had 6 to 8 true leaves with spacing of 0.75 × 1.0 m. Plots were hand-weeded, un-fertilized, and irrigated weekly.

The time for different developmental events (phenophases) was evaluated weekly for the vegetative phase and reproductive phase (flower initiation, flowering, fruiting, and seed maturation) from 10 plants of each population.

Chemical Characterization

Samples of 6 rhizomes from each population were pulverized in a knife mill with a 180 mesh particle size. For extraction, 100 mg samples were weighed in a round bottom matrass, 20 mL ethanol 50% was added, and samples were centrifuged at 5000 rpm for 10 min. Extracts were diluted to 50 mL volume with methanol in a gauged matrass. To determine total phenolic derivatives, gallic acid monomeric precursor was used as marker with a calibration curve of gallic acid as standard between 1–12 mg L^{-1} of final volume. Quantitative analysis was performed in samples from P1 and P2 populations and a blank control (Table 1).

Molecular Characterization

Ploidy levels in *G. quellyon* populations were determined using 1 to 2 cm length roots, obtained from seedlings. For molecular characterization, primordia were collected from leaves of P1 and P2 accessions.

Extracted primordia were placed in 2 mL polypropylene tubes, freezed in liquid nitrogen and stored at –20°C until analysis.

RESULTS AND DISCUSSION

Germination Assay

Significant differences were observed in maximum germination of seeds from both *G. quellyon* populations. Germination percentage increased when seed received light during germination (Table 2) at 20°C for P1 populations, while P2 seed germination percentage increased at 12°C. Thus, accessions collected from higher altitudes (P1) require higher temperatures to germinate than populations from lower altitudes (P2). P1 seeds did not germinate at 5°C; maximum germination was reached at 20°C (Table 2). In contrast, maximum germination of P2 was 76.5% at 5°C, and 92.5% at 12°C. Similar results had been obtained in seeds of *G. urbanum* and *G. rivale* (Graves and Taylor 1988). When P1 seeds were subjected to a temperature gradient bar between 5 and 35°C, optimum germination temperature was 23.3°C; minimum and maximum temperature were 6.5 and 38.5°C, respectively (Fig. 2).

Under prolonged cold winter environment, it is advantageous that seed germination occur during summer, to avoid exposing seedlings to adverse climate conditions. Many high latitude or altitude adapted species, which do not have cold requirement, do not germinate at low temperatures, as a mechanism to restrict seed germination to summer (Graves and Taylor 1988). This appears to be the mechanism present in P1 since seed germination occurs above 6.5°C.

Table 1. Sample and blank composition for determination of flavonoid concentration.[z]

Sample (without $AlCl_3$)	Blank (with $AlCl_3$)
500 µL extract	500 µL distilled water
1.5 mL ethanol 95% (v/v)	1.5 mL ethanol 95% (v/v)
100 µL distilled water	100 µL $AlCl_3$ 10%
100 mL CH_3COONa	100 mL CH_3COONa
2.8 mL distilled water	2.8 mL distilled water

[z]Samples with and without $AlCl_3$ were evaluated in a spectrophotometer UV VIS at 415 nm.

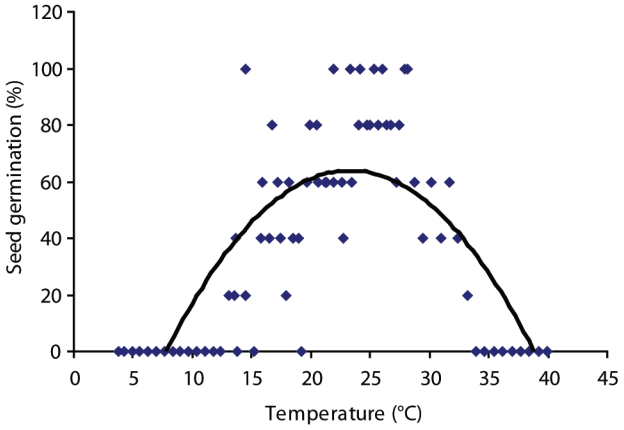

Fig. 2. Minimum, optimum, and maximum seed germination of two *Geum quellyon* populations collected in South Central Chile.

Table 2. Maximum seed germination of two *Geum quellyon* populations collected in South central Chile under two light treatments (with and without light) and three thermal treatments (5°, 12° and 20°C).

		Germination (%)			
Population	Treatment	5°C	12°C	20°C	Mean
	With light	0	21	39	20.0 a[z]
	Without light	0	11	23	11.3 b
P1	Mean	0.0 C	16.0 B	31.0 A	
P2	With light	88.0	98.0	84.0	90.0 a
	Without light	65.0	87.0	72.0	74.7 b
	Mean	76.5 B	92.5 A	78.0 B	

[z]Different uppercase letters among columns and lowercase between rows, indicate significant differences according to Tukey test ($P \leq 0.05$).

Phenology Under Crop Conditions

Both populations differed in phenological behavior. P2 populations collected below 200 m reached reproductive stages the first year of establishment, while P1 accessions did not reached reproductive stages until the second growing season (Fig. 3). P2 budding stage lasted 36 days, 17 days with open flowers, and 22 days to seed production in the first growing season. The second growing season budding stage was shorter, only 10 days, 23 days for open flower, and 28 days to seed production. P1 budding period lasted 15 days, open flower 20 days, and 35 days to seed production from anthesis (Fig. 3)

Chemical Characterization

There were differences in total polyphenol concentration between populations, expressed as gallic acid equivalents (GAE) (Fig. 4). The presence of astringent phenolic compounds in plants has been postulated as a quantitative defense mechanism against herbivores.

Molecular Characterization

Both populations (P1 and P2) had chromosome numbers of $2n = 42$ confirming results of Raynor (1952). Chromosome size was < 3 µm; most chromosomes are metacentric (Levan et al. 1964). *G. quellyon* populations did not show difference in ploidy level. DNA amplification was achieved and fragments approximately of 400 bp were separated and electrophoretically observed in agarose gel (1%) in TBE with ethidium bromide stain (Fig. 5).

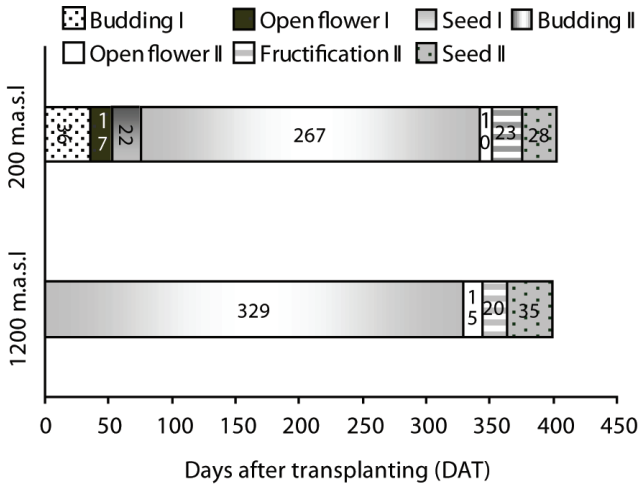

Fig. 3. Vegetative stage length (bud), anthesis, and seed maturation of two *Geum quellyon* populations collected at 200 and 1200 m.a.s.l. Phenological stages correspond to first (I) and second (II) growing season.

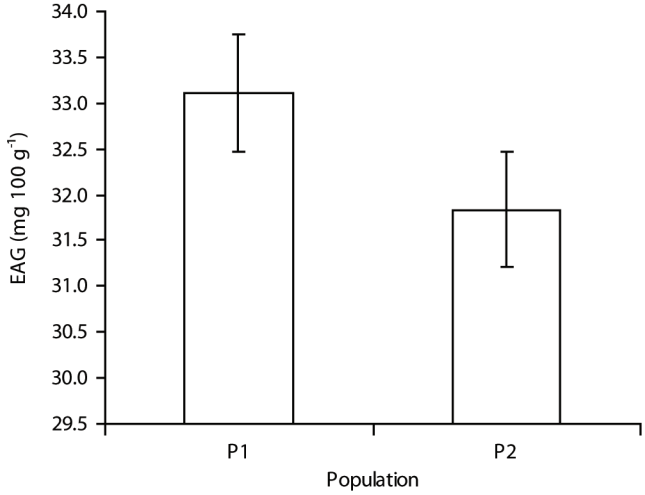

Fig. 4. Total phenol content, expressed as gallic acid equivalents (GAE) in roots of two populations of *Geum quellyon* collected in South central Chile. P1: population collected above 200 m.a.s.l.; P2: population collected under 200 m.a.s.l.

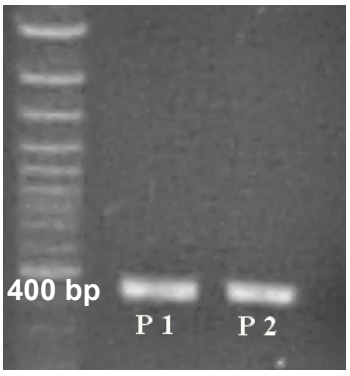

Fig. 5. Gel PCR amplification in the ITS (Internal Transcribed Spacer) region of two populations of *Geum quellyon* collected in South central Chile. P1: population collected above 200 m.a.s.l.; P2: population collected under 200 m.a.s.l.

REFERENCES

Araneda, P. 2003. Validación del uso tradicional de *Geum quellyon* Sweet en la disfunción eréctil masculina. Tesis para optar al grado de Magíster en Ciencias Farmacéuticas, Universidad de Concepción, Chile.

Barbour, M.G., and C.H. Racine. 1967. Construction and performance of a temperature-gradient bar and chamber. Ecology 48:861–863.

FIA. 2001. Estrategia de innovación para la producción de plantas medicinales y aromáticas. Fundacion Para la Innovacion Agraria, Santiago, Chile.

Fox, D.J.C. and P.A. Thompson. 1971. A thermo-gradient apparatus designed for biological investigations over controlled temperature ranges. J. Expt. Bot. 22:741–748.

Gajewski, W. 1959. Evolution in the genus *Geum*. Evolution 13:378–388.

Graves, J. and K. Taylor. 1988. A comparative study of *Geum rivale* L. and *G. urbanum* L. to determine those factors controlling their altitudinal distribution. III. The response of germination to temperature. New Phytologist 110:391–397.

Hoffmann, A., C. Fraga, J. Lastra, and E. Veghazi. 2003. Plantas medicinales de uso común en Chile. 3rd ed. Ed. Fundación Claudio Gay, Santiago, Chile.

Levan, A., K. Fredga, and A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220.

Lodhi, M.A., Guang-Ning Ye, N.F. Weeden, and B.I. Reisch. 1994. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. Plant Mol. Biol. Rptr. 12:6–13.

McCutcheon, A., S. Ellis, R. Hancock, and G. Towers. 1994. Antifungal screening of medicinal plants of British Columbian native peoples. J. Ethnopharmacol. 44:157–169.

Montes, M. s/f. Consideraciones sobre medicina tradicional. Apuntes Botánica y Farmacognosia 2: Plantas medicinales y medicina tradicional. [On line] Universidad de Chile, Facultad de Ciencias Químicas y Farmaceúticas. Biblioteca digital de la Universidad de Chile. Cap. XII. mazinger.sisib.uchile.cl/repositorio/ap/ciencias_quimicas_y_farmaceuticas/apbot-frm2f/montesm02/23.html.

Muñoz, O., M. Montes, and T. Wilkomirsky. 2001. Plantas medicinales de uso en Chile. Química y Farmacología. Ed. Universitaria, Santiago.

Raynor, L.A. 1952. Cytotaxonomic studies of *Geum*. Am. J. Bot. 39:713–719.

Russo, A., V. Cardile, L. Lombardo, L. Vanella, A. Vanella, and J. Garbarino. 2005. Antioxidant activity and antiproliferative action of methanolic extract of *Geum quellyon* Sweet roots in human tumor cell lines. J. Etnopharmacol. 100:323–332.

Smedmark, J., T. Eriksson, R. Evans, and C. Campbell. 2003. Ancient allopolyploid speciation in Geinae (Rosaceae): Evidence from Nuclear Granule-Bound Starch Synthase (GBSSI) Gene Sequences. Syst. Biol. 52:374–385.

Tunon, H., C. Olavsdotter, and L. Bohlin. 1995. Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. J. Ethnopharmacol. 48:61–76.

Vogel, H. 2000. Avances del cultivo de especies medicinales nativas de Chile. Seminario Internacional y Rueda de negocios Plantas medicinales: Mercado, cultivo y procesamiento. Marzo 29–32. Fac. Agronomía. Universidad de Concepción, Chillán, Chile.