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Genetic, Agronomy, and Metabolomics of Prince Edwards Island Wild Rose Collection and Promise for Cultivar Development

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Additional information is available at the end of the chapter

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1. Introduction

Agriculture – the control of plants for human consumption – is believed to have appeared and developed during the paleolithic/neolithic period, ~ 10,000 years ago [1]. The first agriculture had no single or simple origin since a wide variety of plants and animals have been independently domesticated at different times and different places [1-4]. The origin of agriculture and crops domestication is intertwined. Plant domestication involves changes in the plant's genetic makeup and morphological appearance following successive selections within wild plants and based upon on the variations that are best suitable for humans needs [5]. Domestication is therefore an artificial selection process conducted by humans for the production of plants showing fewer undesirable traits compared to its wild related plants, and making them more dependent on the new artificial environments for their continued survival and development. The concept of selection assumes the existence of a population or group of individuals from which choices can be made. Thus, the diversity of morphotypes or genetic diversity is considered as the backbone for plant domestication and crop improvement. Nonetheless, the way this genetic diversity was probed across time has constantly evolved while being a continuum from the first day. Moreover, while the selection criteria for the desired traits and purposes in the ancient domestication process were certainly exclusively based on morphology (size, color, shape of leaves and fruits, easiness for identification) and to satisfy man's energy supply needs (taste and flavour, satiety potential), today, the required traits and purposes for plant domestication (seen as continuum) have been refined and expanded. Indeed, new technologies have been developed for probing the genetic diversity whereas human needs

have increased to include health and wellbeing. As a consequence more specific and defined traits such as a targeted and defined ingredient or metabolite are sought. To date, the pace of plant domestication has slowed down mainly due to the loss of biodiversity but also because of our ability to satisfy our current food needs. Nevertheless, few new crops species are still being introduced into farming system to fill the growing gaps in the need of humans and pets. Although domestication, as a concept, is not the main focus of this chapter (reader can refer to [3, 4, 6-9]), this review will look at some aspects of plant domestication in the 21st century as compared with ancient domestication process, the extent of genetic diversity within North American roses, the challenges associated with the domestication and agronomy of Atlantic Canada wild rose species taken as an example, and how the current biotechnology tools can contribute to an economic crop production.

2. Domestication as a science

2.1. Definition

Domestication was defined by De Wet [8] as "changes in adaptation that insure total fitness in habitats especially prepared by man for his cultigens". Van Raamsdonk [7] refined this definition by taking into account Simmond's [6] observations on plant domestication syndrome because a considerable number of crop plants are dependent on man for establishing new generations due to non-dehiscence, non-shattering, and absence of seed dormancy. Domestication was thus better defined by van Raamsdonk as a process leading to characteristics that are beneficial to humans but generally unprofitable for plants in natural habitats and in the decrease or total lack of capability to disseminate viable offspring [7]. As such defined, the goal for crop domestication appears obvious: setting plant for human's benefits. However, the paths and process followed, and the tools used towards developing a new crop from its wild related plant can greatly vary (Table 1).

2.2. Domestication process and goal

An artificial selection results in a phenotypic evolution [10]. In fact, agriculture started ~10,000 years ago by probing the diversity present within wild plant species and by planting the selected specimens, first in the garden and then in the field setting, a process known as domestication. Although all crops and plant varieties known to man today did not undergo through this classic process (case of known semi-domesticates) [3], the vast majority did go through, and thus being fully or super domesticated [3], depending on era, needs and advances in technology. Domestication is generally considered to be the end-point of a continuum that starts with exploring wild plants, continues through cultivation of plants selected from the wild but not yet genetically different from wild plants, and terminates in the fixation (at some extent), through human selection, of morphological and hence genetic differences distinguishing a domesticate from its wild progenitor. Wild and cultivated populations differ statistically in various characters targeted by human selection, although the cultivated plants may be morphologically indistinguishable from the wild plants [3]. Therefore, cultivated populations

are not genetically fixed for any characters distinguishing them from wild populations, but the frequencies of alleles governing the characters subjected to human selection presumably differ [3]. Casas et al. [11] considered that changes in allele frequencies resulting from human selection constitute at least an incipient domestication, i.e. a nascent domestication. These authors analyzed the morphological variations in wild, managed *in situ*, and cultivated populations of the columnar cactus *Stenocereus stellatus* in central Mexico. They investigated whether morphological divergence has occurred between manipulated and wild populations by the domestication processes. Multivariate statistical analyses showed that individuals grouped according to management options and the fruit characteristics were the most relevant for grouping. Sweet fruits with non-red pulp colors were more frequent in cultivated populations. The fruits were also larger, contained more and bigger seeds, had thinner peel, and fewer spines in cultivated populations than fruits in wild individuals. Phenotypes common in managed *in situ* and cultivated populations generally occur also in the wild but at lower frequencies. However, Gepts [12] considered cultivation as a necessary but insufficient condition for domestication which, at least incipient or semi-domestication, may occur without cultivation by selective removal of undesirable phenotypes and/or enhancement of desirable phenotypes in wild populations [11]. How these different domestication processes and the available tools may apply to wild rosehip is one of the main topics developed in this review.

2.3. Domestication tools

2.3.1. Ancient tools

The oldest cultivated garden rose was *R x richardii* grown and depicted in art works by the Minoan civilization in Crete more than 3500 years ago. Roses were extensively cultivated during the Roman era (625 BC- 476 AD). After the demise of the Roman Empire, the less-appreciated wild-growing roses in Europe and Asia, belonging to *Rosa* section *Canina* and known today as Dogroses were maintained in monasteries for their reputed medicinal properties [13]. By the 18th century, five rose species (*R. gallica*, *R. alba*, *R. damascena*, *R. centrifolia*, and *R. centrifolia moscosa*) sharing a number of features such as double flower, fragrance, flower colour, frost hardiness, spring flowering, resistance to black spot and rust, and susceptibility to mildew had emerged [14]. These five species fall into 5 broad rose classes namely Gallica, Alba, Damask, Centrifolia, and Moss rose, respectively, and referred to as old European roses. These traditional European roses were crossed with roses from China (*R. chinensis*) leading to *Rosa x hybrid*, the modern rose selected for defined traits such as shape, colour and fragrance of the flower bud and flower qualities, stem length, and vase life. During these times, probing the genetic diversity within wild populations and selection of progenies from crosses were solely based on morphology.

2.3.1.1. Probing the genetic diversity

During ancient times, botanists such as Linnaeus [15] have played a crucial role in probing rose genetic diversity and defining boundaries between species. Linnaeus [15] was one of the first botanists to acknowledge the complexity of the genus *Rosa*. In his book "*Species Planta-*

rum” Linnaeus stated that “the species of the genus *Rosa* are difficult to distinguish and determine, I have the impression that nature combines just for fun a number of them and then forms a new one out of the lot, those who have seen only some distinguish them more easily than those who have examined many”. The complexity of the genus has remained enigmatic to taxonomists of the twentieth century [13, 16-19] as the morphological characters are continuous and possibly polygenic making difficult in assigning genotypes that clearly define taxa. Nonetheless, similar to any other plant species, end-uses have been instrumental drivers for probing the genetic diversity and guiding in the selection process.

2.3.1.2. Process and goal for probing the genetic diversity (food and ornamentals)

During the Middle Ages, dogroses were cultivated at monasteries as a medicinal plant and, all parts including rosehips, seeds, petals, leaves and roots were virtually used. Later on in the 19th century, dogroses served as rootstocks to graft modern rose cultivars either as frost or soil born disease resistance sources [13]. They have also been used as a rustic and hardly living fence for fields and public spaces. In the twentieth century, roses have become important horticultural and cosmetic crops receiving much attention from geneticists, breeders, and general public. Hybrid Tea varieties of roses (*Rosa hybrida* L.) are among the most economically important cut-flower plants. The first Hybrid Tea rose was introduced in 1867, and since then more than 10,000 varieties have been released.

The Centre for Variety Research, the Netherlands, has submitted more than 2,800, predominantly Hybrid Tea varieties, for Plant Breeders Rights. This number is increasing annually with 80 applications on average each year. This registration and protection process is based on morphological and physiological characteristics as described by the UPOV (Union Internationale pour la Protection des Obtentions Végétales) guidelines [20]. Wild roses, semi-domesticated and commercial varieties, serve as breeding materials for creating new genetic stocks. These breeding materials generally selected as seed or pollen parents, for flowers that are often fragrant, commonly rose-colored flowers although white or more rarely yellow flowers can be observed in some species [21] are used in crosses. Hence, seedlings of interest with differences in fragrance, colour, shapes, disease resistance genes are selected through extensive field trials and advanced in the registration process [22]. Among the many wild rose species, the selection was obviously based on easy availability, attractiveness of characters, seed set potential, but also the plant morphology such as dwarfness and small size of flowers [22]. During these times less emphasis was made on the wild rose fruit characteristics.

2.3.2. Modern tools

In modern times, these classical methods become less and less efficient as the number of varieties to be tested increases and the genetic distances between varieties becomes smaller [20]. As well, because the needs, objectives, and challenges associated with the rose industry are now changing both in terms of flower and fruit production, combination of morphological, cytological, conventional breeding and biotechnological methods are being widely used for the determination of *Rosa* species as well as for the development of new rose cultivars [23-28].

2.3.2.1. Probing the genetic diversity

Domestication and crop improvement involve the selection of specific alleles at genes controlling key morphological and agronomic traits, resulting in reduced genetic diversity relative to unselected genes [10]. This artificial selection process that operates also in almost all agro-systems, including agroforestry, favours abundance of the preferred targeted phenotypes, and acts with more intensity in household gardens [29]. In the 20th century, probing for crops and their wild relative's genetic diversity has been the focus of extensive investigations. In roses in particular, morphometric [13, 30-34], cytological characters [25, 35] were the most used in the *Rosa* sp taxonomy and phylogeny. But these methods have been proven not to be sufficient in assigning individual genotypes that clearly defined taxa [13]. The 21st century is characterized by a remarkable explosion of molecular tools, highly polymorphic and with high discrimination power, for deciphering differences based on DNA nucleotide sequences. The development of these tools were achieved mostly with the event of polymerase chain reaction (PCR) in the mid 1980's [36], which has revolutionized the field of biology by inspiring the development of many PCR-based technologies, large DNA sequence databases, and increased computer power by bioinformatics. Despite the success of these powerful tools and its speed in advancing our current knowledge of the *Rosa* phylogeny [16, 17, 19, 37-43], there is still not exist at present a single method or tool for tracing a clear cut relative phylogenetic position between *Rosa* subgenera, sections and species within the genus [16], mainly due to low sequence divergence, natural hybridization between taxa, and polyploidy [44]. Rather, complementary methods (morphology, ploidy level, and DNA sequences from both chloroplast and nuclear genomes) using extensive data computing, with iterations and bootstrapping, are now the approach commonly sought [16, 17, 39, 40, 44, 45, 46, 47]. Nonetheless, for well-defined *Rosa* species, the DNA sequence analysis for single nucleotide polymorphism [47] and SSR polymorphism [48] are the preferred choice for distinguishing between genotypes and varieties [20]. The current *Rosa* phylogeny relies mainly on Rehder [49] who subdivided the genus into 4 subgenera: *Hulthemia*, *Platyrhodon*, *hesperhodon*, each with 1 or 2 species, and *Rosa*. Likewise, the large *Rosa* subgenus was divided into 10 sections (*Pimpinellifoliae*, *Rosa*, *Caninae*, *Carolinae*, *Cinnamomae*, *Synstylae*, *Indicae*, *Banksianae*, *Laevigatae*, *Bracteatae*). However, recent molecular evidences do not support distinct subgenera status [16, 50] but did support the presence of 2 main clades. One clade includes subgenera *Rosa* species of sections *Carolinae*, *Cinnamomae*, and *Pimpinellifoliae* (clade 1) and the other clade (clade 2) includes all remaining subgenera *Rosa* sections, excluding the section *Banksianae* which comprises *R. Banksiae* (section *Banksianae*), *R. roxburhii* (subgenera *Platyrhodon*), and *R. persica* (subgenera *Hulthemia*), found to be sister to clade 2 [16]. The section *Caninae* DC forms a large and well-defined group of polyploid taxa and known as dogroses. In this section, pentaploids are the most common, but tetraploid and hexaploids also occur [18]. Bruneau et al. [16] also showed that sections *Cinnamomae* and *Carolinae* form a monophyletic group, and should be merged into one section, referred to as sect *Cinnamomae*. Indeed, section *Cinnamomae* comprises more than 40% of the species in the genus *Rosa*.

2.3.2.2. Process and goal (life quality)

One of the main current questions is whether the process and goal for probing rose genetic diversity has changed over time. Although crop domestication and improvement process is a continuum, it evolves constantly with the available technologies in order to meet and fulfill

the societal needs. In the present global economy, the scale of demands for any good has increased and the trade has become multidirectional (selling in all part of globe) with multiple layers (one product could be found in many other products as additive or supplement) (Table 1). Thus, probing the genetic diversity of a plant species which end-product would satisfy these new needs both in terms of quality, quantity, sustainability and stability has become the new challenge for plant products developers. Hence, the need for well characterized germplasm with stable and preserved genetic identity is becoming the landmark for todays and tomorrow's natural product designers and developers. Therefore, sophisticated molecular tools [51, 52] as well as mass tissue culture and plant propagation tools are being employed to insure stability and sustainability.

	Ancient domestication	Domestication in the 21st century	References
Purposes	Food, medicine clothing, energy, sustainability	Food, clothing, energy, health, life quality, sustainability	[28, 53, 54]
Screening methods	Morphology, taste, flavour, energy	Morphology, genetic DNA markers, QTLs, taste, flavour, energy, metabolite profiles,	[20, 41, 51, 52]
Production paths	Gathering, yards and small farms sowing and harvesting, human and animal force	Experimental tubes, growth chambers, greenhouse and fields, large commercial fields, high throughput management, human and animal force and mechanization	[28, 53]
Purity	Composite	Composite, variety	
Ecosystem	Complex	Simple	[53]
Yield	Low	High	[28, 55, 56]
Value chain	Self, local consumption,	Global, processing, distribution and marketing networks	

Table 1. Comparative pathways of ancient and modern plant domestication processes: purposes, tools, and expectations

3. Plant domestication in the 21st century: A case study with PEI wild rosehips

One of the most recent and successful domestication of a wild species is that of the North American ginseng [57]. Similar to ginseng, interests in wild rosehip products are increasing worldwide due to its nutraceutical and natural health products properties [13]. With aging

and changing eating lifestyles, the incidence of chronic diseases is increasing worldwide. Despite success achieved in fighting these diseases, prevention measures have become top priorities for citizens and public health systems. Recently, increasing interest has been expressed in plant natural products as preventative agents. Hence, plant product preparations such as those from rosehip have been used as food and medicine for centuries. The genus *Rosa* contains more than 150 species. They are widespread in North America within the *Cinnamomae* section and are renowned for the vitamin C content [58-61]. Although formulations from *Rosa canina* have been associated with the treatment and symptom reduction of inflammation and arthritis, the vast majority of wild rose species are fully unexplored for their health potential. To date, most of the reported studies were focused mainly on *Rosa* species within the *Caninae* section which comprises 20 – 30 *Rosa* species known as dogroses [18, 42] and is currently the focus of major domestication research programs for the production and commercialisation of rosehips (fruits) around the world, particularly in Northern Europe, Germany, Turkey, Eastern Europe and Chile [13]. So far, less emphasis has been made on *Rosa* species belonging to *R. carolina* complex within the *Cinnamomae* section and the rosehips production from the eastern North American native wild roses is new and emerging [55, 56]. This section deals with the genetic diversity of PEI wild rosehips, the challenges associated with their domestication as well as the agronomic practices that could ensure an economic production.

3.1. Introduction to the genus *Rosa*

The genus *Rosa* (*Rosaceae*) originated in the temperate regions of the northern hemisphere, including North America, Europe, Asia, and the Middle East, with the greatest diversity of species found in western China, where it is endemic, and is now widespread all over the globe [18]. With this wide distribution range and the high number of species (more than 150 shrub species), the delimitation of the species boundaries remained a challenge for taxonomists and molecular biologists [16, 21, 41, 44].

3.2. *Rosa* species phylogeny and biodiversity

3.2.1. Global *Rosa* species biodiversity and phylogeny

The taxonomy and breeding system of the genus *Rosa* has been recently reviewed by several authors [13, 16, 21, 38, 49, 62, 63] and the reader is invited to find more details in these treatments. Of particular interests are works reported by Werlemark and Nybom [13] and Macphail and Kevan [21] on one hands, and those by Bruneau et al. [16] and Joly and Bruneau [44] on the other hands, focusing on the European Dogroses from section *Caninae* and the North American *Rosa* species from section *Cinnamomae*, respectively. Wild rose species from these two sections are currently extensively investigated for domestication purposes and commercial rosehip production [13, 55, 64-67]. As our interest lies mainly in the domestication of North American wild roses, the next section of this review will put more emphasis on the biodiversity and phylogeny of wild rose species commonly encountered in this part of the globe and more specifically in Canada, a country as large as the whole Europe (West and East taken together, excluding the former USSR).

3.2.2. North American *Rosa* species biodiversity and phylogeny

Biodiversity of the North American wild roses has been investigated by botanists in the early 1900's. Watson [68], Crepin [69, 70], Erlanson MacFarlane [71, 72] have described and defined 13 - 22 *Rosa* species in North America. This important polymorphism in *Rosa* species, especially in eastern North America, together with hybridization and polyploidy have long been considered as the major causes of taxonomic confusion in the genus [17]. Alfred Rehder (1869-1949) established the first foundation of *Rosa* species taxonomic relationship in a book entitled "*The Manual of Cultivated Trees and Shrubs Hardy in North America Exclusive of the Subtropical and Warmer Temperate Regions*" published in 1940 [49]. Rehder provided concise physical description, time of flowering, region of native habitat, hardiness zone, distinguishing features and pertinent information on North American roses, and subdivided the genus *Rosa* into 4 subgenera and 10 sections, including the *Rosa carolina* L. complex of section *Cinnamomae*. East of the Rocky Mountain, the *Rosa Carolina* complex is composed of five diploid species (*R. blanda*, Ait., *R. foliolosa* Nutt., *R. nitida* Wild., *R. palustris* March., and *R. Woodsii* Lindl.), three tetraploid species (*R. carolina* L., *R. virginiana* Mill., and *R. arkansana* Porter) and one hexaploid/octaploid species (*R. acicularis* Lindl.) which is morphologically distinct from all other species [17]. The taxonomic problems are well known at the diploid level, where some species hybridize and are also morphologically difficult to distinguish (which is particular true for *R. blanda* and *R. woodsii*), but are even more acute at the polyploidy level. *Rosa carolina* which is widespread East of the Mississippi river hybridizes with *R. Arkansana* in the western part of its distribution [71] but also in the East with *R. virginiana*. Moreover, the morphological similarity cuts across ploidy levels and no single morphological character can be used to distinguish one species to another [17]. Thanks to molecular tools (AFLP, SNP), haplotype network analysis using statistical parsimony, genealogical approach, and multivariate analysis of 25 morphological characters including ploidy determination based on stomatal guard cell lengths, Joly et al. [17] and Joly and Bruneau [44] determined four species at the diploid level and that were separated into 2 groups in the east of the Rocky Mountains: one group consists of *R. blanda* - *R. woodsii* (which were indistinguishable and should be considered as a single species), and the other group is consisted of *R. foliolosa*, *R. nitida*, and *R. palustris*. The authors also determined 3 species at the polyploid level: *R. arkansana*, *R. carolina*, *R. virginiana*, with evidence of hybridization between them. The diploids that are involved in the origins of the polyploid species in that region were also proposed. For Joly et al. [17], only diploids east of the Rocky Mountains are involved in the origins of polyploids. *Rosa arkansana* is derived from the *blanda-woodsii* group, *R. virginiana* originated from the *foliolosa-nitida-palustris* group, and *R. carolina* is derived from a hybrid between the two diploid groups. Thus, for wild rose species domestication and commercial production purposes in the Canadian Maritimes where both North American native wild species of the *R. carolina* complex grow in sympatry and also along with naturalized species such as *R. rugosa* or other members of dogroses (Figure 1), a careful species determination as well as genotypic identification of collected germplasm for propagation are of critical importance to ensure, genetic purity and traceability.

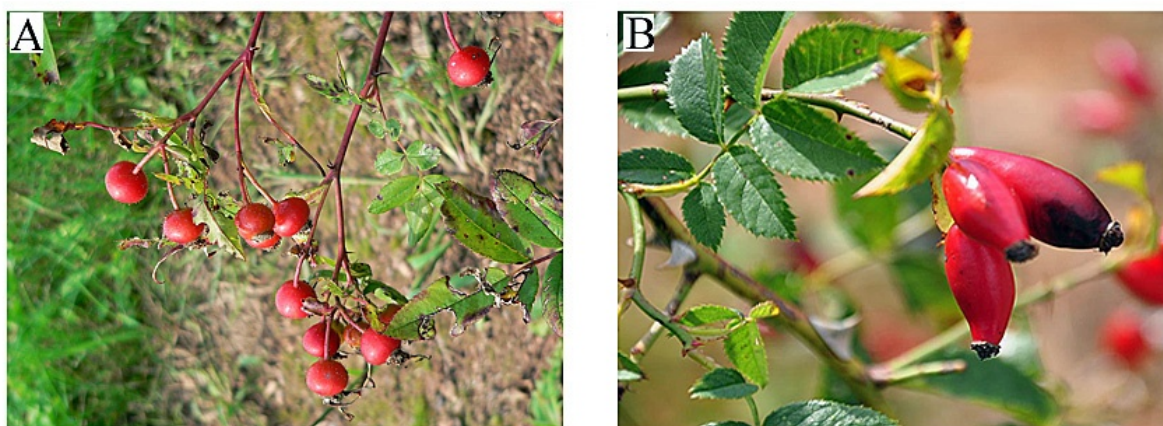


Figure 1. Diversity of rosehip morphology in the Atlantic Canada landscape. A, typical morphology PEI grown rosehip. B, morphological feature of a naturalized rosehip to Atlantic Canada.

3.2.3. Genetic and Metabolite diversity within the Prince Edward Island's field collection

Using SSR markers [20] and single nucleotide polymorphisms analysis, our group has assessed the genetic diversity within 30 ecotypes under cultivation and identified three major clusters, with cluster 2 and 3 showing 2 and 3 sub-clusters, respectively [65, 73]. The metabolite profiles in the flesh, seed, and fuzz for anthocyanins, flavonols, tilirosides which is a potent antidiabetic compound, tannins and fatty acids were also determined from the 30 ecotypes [65, 73]. The level of anthocyanin was very low in all ecotypes, with only one ecotype showing a level that was 30-40 % higher compared to the average. A large diversity was observed for flavonols and tiliroside among ecotypes. Only 4 ecotypes had a high content for both flavonols and tiliroside in the analyzed tissues (Ghose et al, submitted). One ecotype showed 18:3 level as high as 41.2%. The data suggests that it is possible to select and propagate a given ecotype for its unique metabolite profile for commercial and drug production [65, 73].

3.3. Domestication and end uses

Roses have been domesticated by man first for the beauty of their flower and incorporated in many cultural and political practices [74] and are now encountered on all continents, climates, and market places. Nonetheless, the medicinal uses of rose leaves, flowers and fruits were also widespread in human history [13, 54, 75-78].

3.3.1. Flower roses

The best known uses for roses are their flowers as ornamental on tables, in home backyards, public gardens and spaces. Historically, only very few wild rose species (at most 5 to 11 species) have been involved as parents in the today flower roses. One example of using native rose species in North America is related to the Parkland Rose series developed at AAFC in Morden, Manitoba. These flower roses are hardy, winter resistant and some of these rose varieties involve in their

pedigree *R. Arkansana* which is encountered east of the Rocky Mountain in Canada. Beside, its ornamental features, rose flowers are valuable for the cosmetic industry [75, 76, 78].

3.3.2. Wild rosehips

The fruits of roses, the hips, have been highly regarded as important food and medicinal sources [13, 54, 79]. Rosehip is appreciated as traditional vitamin C rich soup in Sweden where the demand is particularly high [80]. Its flesh and seeds have been used in concoctions and tonics for various ailments, including the use as laxative and diuretic, against common cold, gastrointestinal disorders, gastric ulcers [77, 81, 82], and anti-inflammatory diseases such as arthritis [83]. A review on the major chemical components of dogrose hips from was recently made by Werlemark [13]. However, a marked variation in chemical composition is associated with species, genotypes, and environments in which the plants evolve. For example, Melville and Pyke [84] found a weak correlation between latitude and vitamin C content of British rosehip populations from Scotland and England. Similarly, Werlemark [13] hypothesised that rosehips produced in a colder climate, especially with colder summer, may have higher vitamin C content compared to those that have been maturing in a warmer climate and also anticipated that local variations in precipitations and temperatures during summer may affect the chemical content of rosehips. It is reasonable to assume that, with different species and cooler summer and fall (Table 2), the Canadian Maritime wild rose species would show different chemical composition, especially in terms of relative amount when compared to their European and South American counterparts. By comparing some rosehip samples from Prince Edward Island, Denmark, Chile and South Africa, our group observed differences between origins, especially with regards to total oil content and fatty acid profiles (Figure 2). Nonetheless, sample preparation (harvesting time and conditioning) can also be a major source of variation. It will be of interest to compare the chemical composition of rosehips collected in each of these regions during the same summer or fall for obtaining factual and conclusive answers to these assumptions.

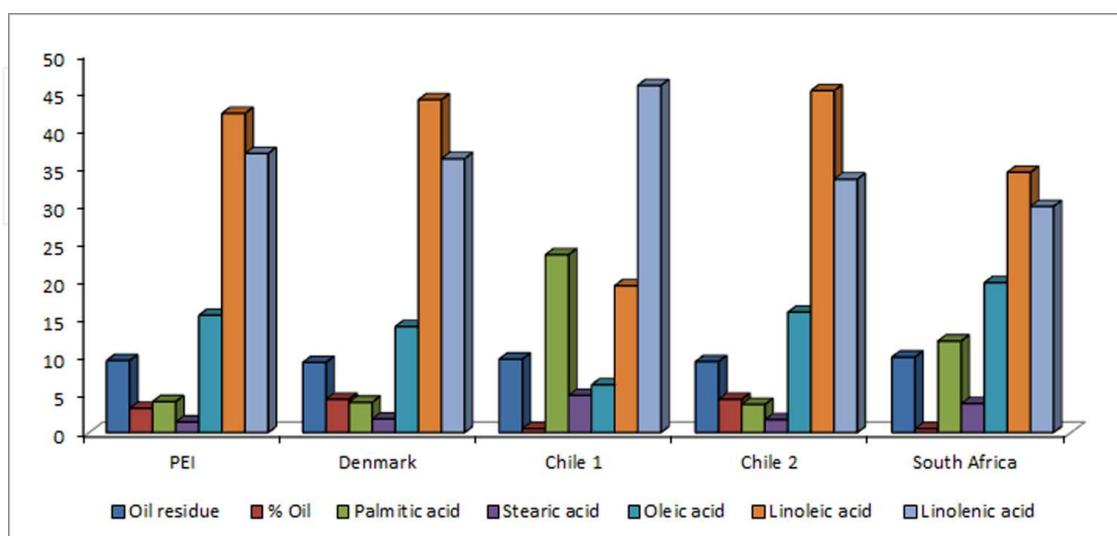


Figure 2. Comparative study of rosehip samples from Prince Edward Island, Denmark, Chile, and South Africa.

Rosehip seed contains pretty well balanced omega-6 (18:2) / omega-3 (18:3) fatty acid ratio and also shows relatively high level of oleic acid as compared to olive and canola oils that are rich in oleic acid but low in both linoleic and linolenic acids (Figure 3). As genetic variability for fatty acid composition has been observed in PEI wild roses (Ghose et al, submitted) and the seed oil content is relatively low, breeding efforts could contribute to increase the oil content.

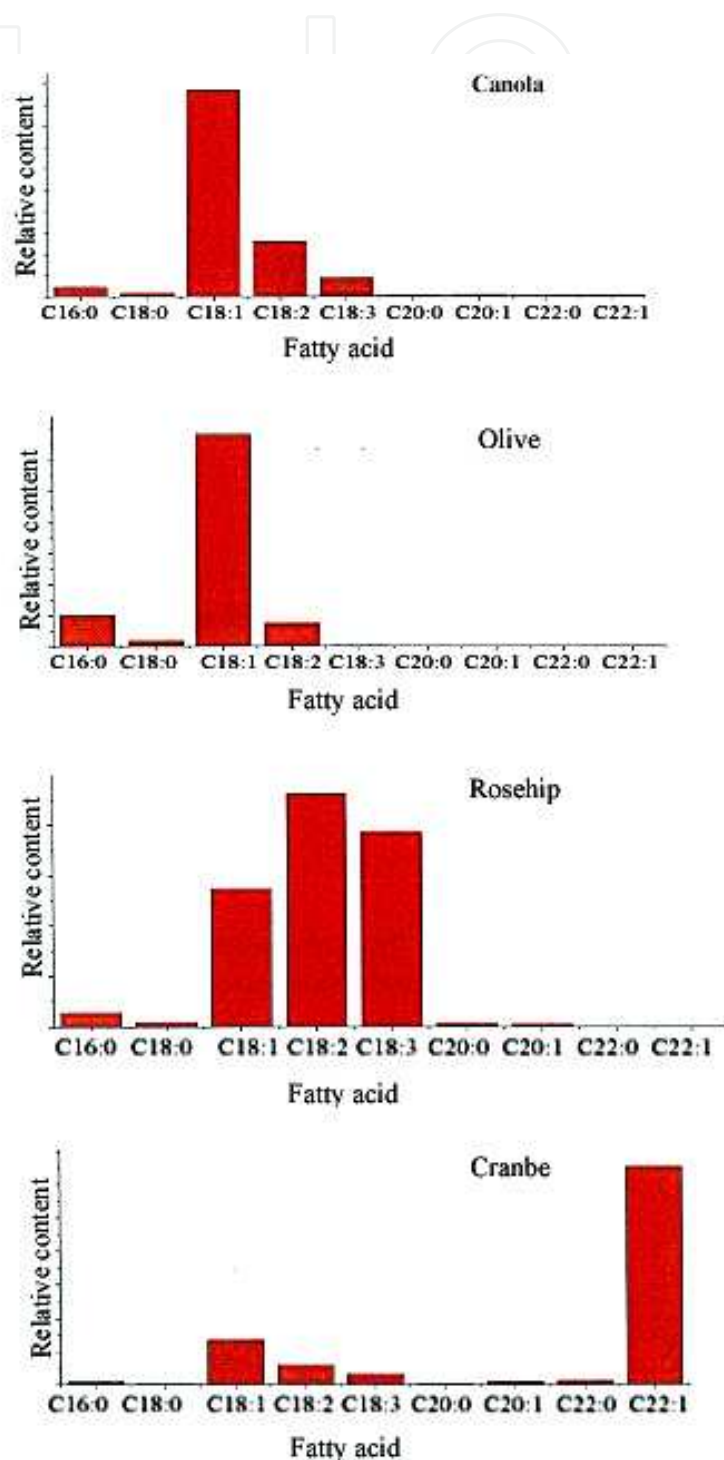


Figure 3. Comparative fatty acid profile of rosehip with three oilseed crops.

3.3.2.1. Agronomy

Although a high value was recognized to rosehip throughout centuries, it is only recently that the wild roses are being domesticated and cultivated for their fruits and to develop agronomic practices that ensure an economic production of the hips [28, 51, 52, 56, 77, 85]. However, due to the diversity of species, genotypes, soils and climates, different agronomic practices are being implemented and tested in different regions, including Denmark, Turkey, Bulgaria, Chile and Canada. Whereas Chilean started their trials by developing a nursery built on the "Tunnel" greenhouse model with a capacity to accommodate 15.000 cuttings, under an irrigation system with nebulizers to reduce temperature and humidification before a developmental stage in the fields, the Danish, Swedish and Canadian choose to established field trials using wild cutting, spacing, density and nutrient management trials [28, 55]. In Sweden, the germplasm used were mostly concentrated on the Scandinavian *Rosa* species of section *Caninae* especially, *R. dumalis*, *R. rubiginosa* and their interspecific hybrids [86] whereas Danish rosehips are produced mainly from *R. canina* (www.hyben-vital.com) although it may also involve other Scandinavian species. In Chile, the current production is mainly focused on wild hand-harvested hips from uncharacterized and naturalized species introduced to south America by Spanish and is mostly a mixture of *R. rubiginosa*, *R. canina*, *R. moschata* and many other species found in western Europe [66]. In Prince Edwards Island, (Canada), current recent genetic study based on 30 wild ecotypes collected from this province suggested that all accessions currently under field trial are from *R. virginiana* and its natural hybrids with *R. Carolina* (Ghose et al, submitted). At present, very few cultivars have been named and released for commercial fruit production. One cultivar, the cultivar "Mechthilde von Neuerburg" derived from *R. rubiginosa* was reported in Germany. Two cultivars (Sylwia and Sylwana) derived from *R. canina* were reported in Poland, whereas cultivar Plovdiv 1 from *R. canina*, and cultivar Karpatia from *R. villosa* were reported in Bulgaria and Slovakia, respectively [13]. For all of these semi-domesticated wild rosehips, it is not known or reported whether the ongoing domestication process has already impacted on some of the phenotypic traits such fruit size, fruit setting or metabolite profile. By comparing the pomology characteristics of 5 wild rosehip ecotypes growing in the wild or in the field settings, we observed that the field setting contributed to increase the fruits size and delayed the maturity when compared with growing in the wild, suggesting an occurrence of a domestication syndrome for these traits (Fofana, personal observation). However, no significant difference was found between the two environments for the number of seed in each of the ecotype.

3.3.2.1.1. Soils and climates

Although originally native to temperate regions of the globe, roses have adapted to warmer regions and grow well now in very diversified habitats and soil types [13, 79]. The soil should be well drained though and not heavy. Species preference for soil type has nonetheless been reported. *R. villosa* was reported to grow better in a dry soil with low calcium content whereas *R. canina* and *R. dumalis* prefer more calcareous soil. *R. rubiginosa* also prefers more calcium and grows well in a relatively heavy soil [13]. *R. palustris* grows in marshes and *R. nitida* in bogs. Similarly, *R. virginiana* likes salt marshes and salty soils (Joly, personal communications).

In Prince Edwards Island province (Canada), wild rosehips are found in a variety of habitats including hedgerows, wet and dry pastures, thickets, swamps and uplands in dry orthic humo-ferric Podzol sandy soils [55]. In hard winter climates such as Canada, plant survival rate in the field setting can vary from genotype to genotype and for the same genotype, plastic coverage has been shown to increase the winter survival rate (Figure 4).



Figure 4. Effect of planting beds coverage with plastic on winter survival.

	Temperature (°C)		Precipitations (mm)		Soil type	Latitude
	Summer	Fall	Summer	Fall		
PEI Canada	16 – 22	7 – 18	270	300	Orthic humo-ferric Podzol with sandy loam	46.04 – 46.57
Denmark	17	9	170	150	Typic Fragiudalf	55 – 57.4
Sweden	13	5	180	120- 140	Aeric Endoaquept	55 – 68 N
Turkey	17 – 29	6 – 7	50	70	Typic Haploxeroll	36 – 42 N
Bulgaria	25	14	180	120	pseudopodzolic- podzolic	41 – 43 N
Chile	17 – 28	8 - 20	350- 500	200 -300	Andisol - Ultisol	18 – 58 S

Table 2. Comparison of average temperature and precipitations during summer and fall in major rosehip production countries.

3.3.2.1.2. Fertilization

Barry et al [55] described the first time the establishment of field trial for North American wild roses belonging to the *R. carolina* complex, with as an objective to investigate the effects of several field management practices on commercial rosehip production in Atlantic Canada. Treatments were applied at planting in a factorial randomized complete block design in June 2004 and included three in-row mulch (none, bark, and straw) treatments, three in-row fertility

(none, compost, and fertilizer) treatments, and two interrow management (tilled and sod) treatments. The compost consisted of an initial mix of softwood sawdust, lobster waste, and old hay. Prior to planting, compost was applied at 60 t ha⁻¹ (54 kg plot⁻¹) in a 1-m band over the row and was incorporated by hand raking. The fertilizer used was a commercial grade (5N-20P-20K). This fertilizer formulation was chosen for use during the first year to promote root development and plant establishment. During the second year (2005), compost was reapplied as top-dress on 22 June 2005 and the fertilizer used was a commercial grade (10N-10P-10K), which was applied as top-dress on 25 May 2005. A fertilizer with higher nitrogen content was chosen with the aim of improving overall plant health and yield during the second growing season. Fertilizer was applied at a rate of 800 kg ha⁻¹ (648 g plot⁻¹) in a 1-m band over the planting row. In Dogroses, Werlemark and Nybom [13] reported 50 g NPK for each plant at planting and 300 kg/ha of organic-mineral NKP in the subsequent year, with additional calcium amendment depending on soil types and species. In Prince Edwards Island, mulching increased nutrient uptake of N and P and increased plant growth. Fertilizer increased plant growth and yield of rose hips compared to no fertilizer or compost treatments. Tilled interrow treatment increased in shoot lengths, diameters, and plant spreads compared to interrow sod. The study indicated that during the early establishment years of a rose hip plantation in Atlantic Canada, wild roses grow best with the use of mulch, fertilizer, and tillage between the rows [55].

3.3.2.1.3. Pests and diseases management

Traditionally, fungal diseases such as black spot caused by *Diplocarpon rosae*, powdery mildew (*Podosphaera pannosa*) rusts (*Phargmidium spp*) and leaf spot (*Sphaceloma rosarum*) have been reported to be problematic in ornamental roses [87-90] and field-grown dogroses [13, 48, 91, 92]. These fungal diseases management is carried through fungicide treatment [93] and selection of genetic resistance [94-96]. Genetic resistance sources within wild rose species within *Caninae* section have been investigated for field rosehip production. Fungal disease tolerance characteristics were identified in *R. rubiginosa* and in interspecific hybrids involving species from *Caninae* and *Cinnamomae* sections [48]. Up to date, no such disease resistance screening has been performed within the *R. carolina* complex for a commercial wild rosehips production in North American. However, our observations in the field showed evidence of these diseases on PEI wild roses (Figure 5). Research in this field should be carried to mitigate the disease incidence in their new field environment. As for any crop, introduction of elite genotypes in cropping systems for rosehips production will lead to a decreased genetic diversity of the cultigens. It is thus anticipated that more susceptibility to major diseases could be observed in the field as compared to the wild populations from which they derive. The preservation of natural habitats hosting the wild populations is of great importance to ensure an availability of genetic stocks to be used in the introgression of disease resistance genes from the wild types to the cultigens.

Insects such aphids (*Aphidina*), grasshoppers (*Orthoptera*), mites (*Tetranychidae*), sawflies (*Tenthredinidae*), gall-making cynipids (*Diplolepis*) as well as the rosehip fly (*Rhagoletis alternate*) have also been reported in dogrose orchards and to cause severe damage in some cases

[13]. Nematode (*Pratylenchus penetrans*) is causal pests of severe lesions to roots in a wide range of ornamental hosts, including roses, mainly in temperate regions. Peng [97] reported that *R. virginiana* is a good nematode resistance source. Because Prince Edwards Island is world leading potato producing area with prevalence of nematodes in the agricultural landscape, development of rosehips orchards with *R. virginiana* genetic background could be a mean for reducing nematode populations in highly infested fields.

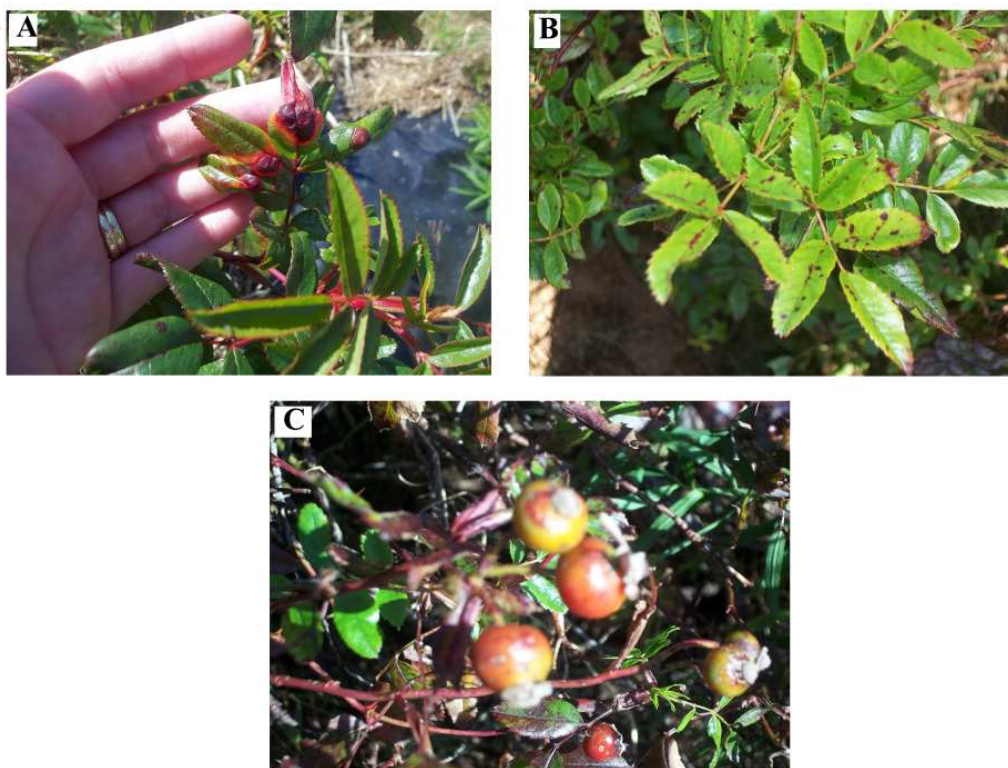


Figure 5. Foliar and fruits diseases in wild roses. A, powdery mildew; B, leaf spot; C, lesions on immature rosehips probably caused by *Phargmidium spp* (Rust) or *Sphaceloma rosarum* (leaf spot).

3.3.2.1.4. Yield and storage

Rosehip yield vary considerably depending on the plant material, cultivation procedures, age of orchard, and harvesting methods. Werlemark and Nybom [13] reported that up to 8 kg of rosehips per bush could be harvested by hand in commercial planting of dogrose hybrid PiRo 3. Similarly up to 3 t/ha could be obtained from *R. dumalis* and *R. rubiginosa* with mechanical harvesting in Sweden. In these cases however, no mention is made about the age of the orchards as yield increases markedly several years after planting. In contrast, Sanderson and Fillmore [56], reported in 14 rosehip ecotypes of the *R. Carolina* complex grown in field condition an average rosehip yield ranging between 411 and 2000 kg/ha, with a fruit mean weight of 1.01 – 1.62 g, over the first four hand harvesting years. The lowest and highest yielding selections showed 910 and 3634 kg/ha in the fourth years, respectively (Table 3).

Compared with reports by Ercisli and Guleryuz [98], Dogan and Kazankaya [99], Güneş and Dölek [100], the fruit weight reported by Sanderson is lower but showed relatively narrow range of variation between ecotypes, reflecting the relatively narrow genetic diversity among these ecotypes. Joly (personal communication) reported that *R. virginiana* and *R. Arkansana* are the two species with the greatest number of fruits per flowering branches. They have more fruits than *R. carolina* and the height of *R. virginiana* makes it one of the most productive North American roses. To preserve the integrity of rosehip bioactives, the postharvest handling and storage conditions are key factors. Both sun-drying and mechanical dryers are being used at commercial scale and the reader can see more details in Werlemark and Nybom [13].

Selection	Biological yield					Mean fruit weight (g)
	2006	2007	2008	2009	Mean	
	(kg ha ⁻¹)					
s26	877	1413	2368	3634	2000	1.62
s30	347	569	1557	2676	1431	1.31
s28	498	335	1136	1759	946	1.57
s22	416	422	831	1464	783	1.29
s67	270	338	910	1440	740	1.39
s25	355	116	562	1725	719	1.29
s57	195	371	941	1178	675	1.03
s33	395	330	654	1227	657	1.33
s55	313	384	679	1167	638	1.42
s36	181	166	862	1307	622	1.01
s140	300	186	576	1342	610	1.21
s142	406	430	464	956	568	1.17
s68	284	281	430	1092	514	1.21
s122	246	83	416	910	411	1.12
Grand mean	363	387	885	1563	808	1.28

Table 3. Yield progression over four years after plantation and mean fruit weight of 14 rosehip ecotypes grown in field (2006-2009)

3.3.2.2. Biotechnology

One of the shortcoming issues for the establishment of commercial rosehip production orchard is the availability plant materials for large acreages. So far, all established fields are based on cuttings or seedlings obtained from wild selections. Because of the genetic diversity within the genus *Rosa* and morphological similarities between species, hybrids (interspecific and intraspecific) and their parental species at the collection sites, an accurate identification at the collection site and the traceability of the putative cultivars under development is challenging and not guaranteed. This issue will become major issues in a near future as rosehip provenances will increase and the bioactive metabolites that are associated to each species, provenance, and ecotypes are made available for marketing purposes. Thus, the use of combined

morphological, cytological, and molecular biology tools for assigning a genetic identity, and the use of regeneration technologies that ensure mass plant production and ensuring the genetic integrity of clones is a research direction that should be undertaken similarly to the ornamental flower industry.

3.3.2.2.1. Regeneration and propagation

3.3.2.2.1.1. Regeneration by seed

The use of plant regeneration from seed for commercial production has been reported [85, 101]. It ensures the production of higher number of plants for field planting in a relatively short period of time. However, the mating system of *Rosa* species is a major source of genetic variability between plant materials obtained using such an approach, especially when the seed is collected from uncontrolled sources like wild plants.

3.3.2.2.1.2. Cutting and explants

Cuttings and explants are currently the materials of choice in commercial wild rose production [64, 86, 101], and most, if not all, of these explants (Figure 6) are derived from wild plants. Wild rose plants grow in the nature as populations that can involve different species, interspecific and intraspecific hybrids, parental and sibling all growing in a confined area. Collecting cuttings in such an environment, even from the same patch, does not ensure the genetic integrity of the collected material for propagation. Once collected, the material should be well characterized and identified. Now, remains our ability to get enough characterized plant materials for large field planting. We believe that the well characterized plant material should be used as starting point for plant regeneration and mass production in the form of rooted seedling or cuttings. This is the approach we pursue in Canada for commercial wild rose production (Figure 7).

3.3.2.2.1.3. Tissue culture

Tissue protocols have been developed and available for flower roses [102-104] and could be applied to rosehip production. Once elite genotypes such as those reported by Sanderson and Fillmore [56] are identified, tissue culture should be able to ensure a sustainable plant production or field planting by growers (Figure 7).

3.3.2.2.2. Cell culture

Similar to tissue culture, rose plants can be regenerated by cell culture. Contrary to tissue culture however, the new plants are obtained from callus generated from sterile explants. This method leads to pure line but can also create new lines different from the mother plant from which the explant was obtained because of somaclonal variations that may occur during the induction of callus and regeneration processes. Thus, for the production of mass plant production from a selected elite wild ecotype, tissue culture appears more appropriate as it minimizes the risk of somaclonal variations while showing high rate of plant multiplication.



Figure 6. Rose cuttings for multiplication. Sterile rose dormant stems were conditioned to break dormancy. Note the active buds sprouting.

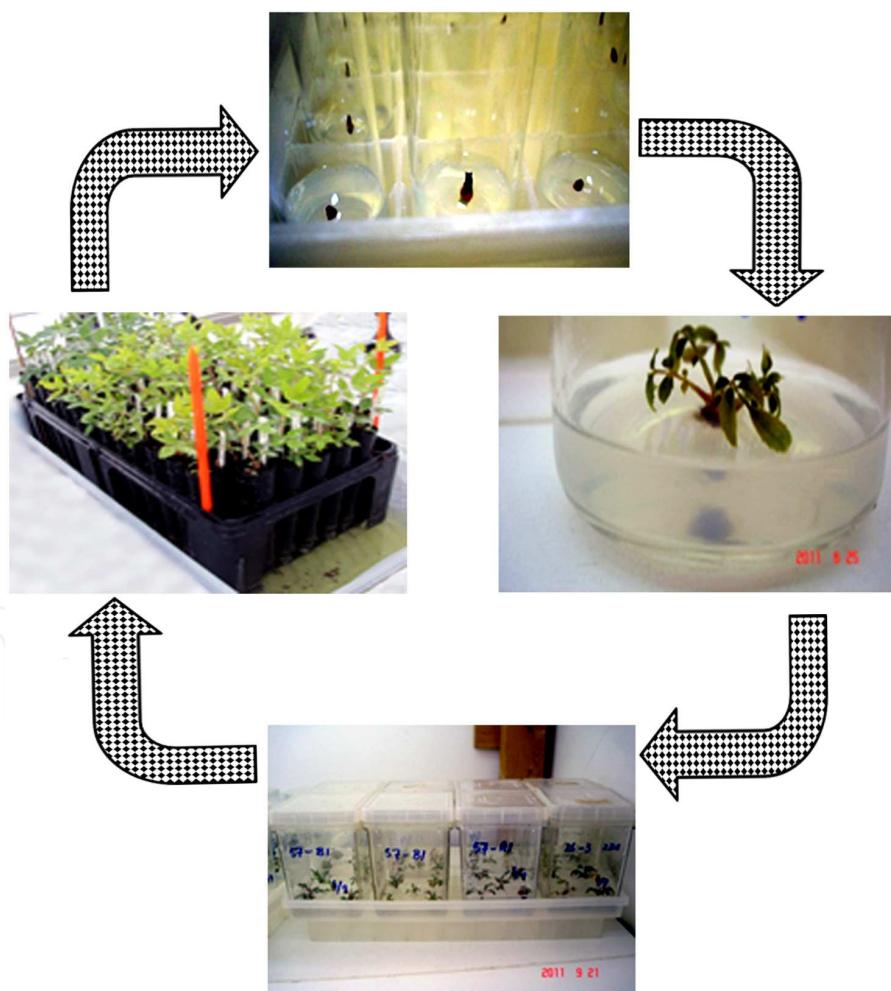


Figure 7. Mass rosehip plant regeneration from active buds of well characterized rosehip genotypes. A, active buds in regeneration media; B, regenerated rose plant; C, plant multiplication in rooting media; and D, acclimation in greenhouse.

4. Conclusions

With the increasing demands for natural health products, plant biodiversity is being thoroughly revisited. The genus *Rosa* has a complex taxonomy that is still being investigated with scrutiny. East of the Rocky Mountain, *Rosa* species belonging to *R. carolina* complex in the *Cinnamomae* section include five diploid species, three tetraploid species and their natural hybrid. Several of these species as well as their interspecific hybrids are encountered on Prince Edwards Islands, Canada. A commercial rosehip production program using wild selected ecotypes has been developed and elite selections with high yielding potential have been identified and agronomic practices set for field management. The collection has been characterised using a combined morphological, cytological and molecular tools and appears to be made of *R. virginiana* and its natural hybrids with *R. carolina*. Genetic and metabolite diversity among these wild ecotypes was observed and could be of high potential for large field production and breeding programs. However, the disease resistance status in this complex is unknown. As for any new crop, increased incidence of existing diseases and recruitment of new diseases is anticipated in the field setting as compared to the wild populations from which they derive. The preservation of natural habitats hosting the wild populations as source of genetic stocks is critical to ensure gene transfer from the wild types to the cultigens through breeding. Future works should also aim at developing mass plant production to ensure sustainable plant material supply from the elite selections.

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