Rubus chamaemorus L. – a boreal plant rich in biologically active metabolites: a review

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Abstract: Cloudberry, Rubus chamaemorus L. (Rosaceae), is a boreal, herbaceous plant with a circumpolar distribution. In Poland, it is under strict protection as a glacial relict. This paper presents a brief review of the biology and distribution of this species, as well as the chemical composition of the fruit. The primary compounds of interest are vitamin C and ellagotannins, especially the high level of ellagic acid, which exhibits bioactivity. In vitro systems for micropropagation of cloudberry and production of encapsulated shoot buds as well as medium-term storage of shoot cultures appear feasible, and may find application in conservation of Rubus chamaemorus in Poland and other countries. The review is focused on botanical characteristics and reproduction process in cloudberry, in order to develop a rationale for an in vitro system for both micropropagation and production of bioactive compounds.

Key words: Rubus chamaemorus, biology, distribution, secondary metabolites, in vitro

THE PLANT AND ITS DISTRIBUTION

European species of the genus Rubus L. (Rosaceae) belong to 4 subgenera. In the subgenus Rubus, about 2,000 taxa have been described, but most of them are of little taxonomic and economic importance (WEBER 1999). By contrast, the subgenus Chamaerubus O. Kuntze includes only one but valid and important species – Rubus chamaemorus L. (WEBER 1995). Its vernacular names are: cloudberry (English), molte (Norwegian), Moltebeere (German), hjoiron (Swedish), hillan (Finnish), multebaer (Danish), malina moroszka (Polish), морошка приземистая [moroshka prizemistaya] (Russian), ostružinik (Czechič) (ØSTGÅRD 1964, MÄKINEN & OIKARINEN 1974, WARR et al.1979, RAPP 1991, WEBER 1995).

R. chamaemorus is an octoploid (2n=8x=56), dioecious, herbaceous perennial (Fig. 1). Its vegetative and flowering shoots are 5–25 cm high, erect, and more or less glandular, arising from creeping rhizomes. The annual shoots usually produce one to four leaves with lamina 2–5 cm long and 3–7 cm broad. They are simple, deeply cordate at the base, reniform, rugose, and have 5–7 serrate lobes. Glandular
hairs occur mainly on the leaf underside and cover rather thickly petioles, which are 1–7 cm long. Young leaves are bright-green, while mature ones are dark-green. Flowers are solitary, terminal and strictly dioecious. Male and female flowers are similar in their general appearance. Male flowers open earlier and secrete abundant nectar, while female flowers produce it in very small amounts. In male flowers, when the corolla drops off, the calyx persists and spreads out flat, whereas in female flowers the calyx surrounds the young aggregate fruit. Usually 5 (or more) petals are 8–12 mm long, obovate, white, hairy, and much larger than 5 sepals. The pedicel and calyx are covered with short-stalked glands. The aggregate fruit consists of 4–20 edible drupelets, which are red when unripe, turning soft orange (amber-coloured) at maturity. Each drupelet contains a smooth, hard stone. The number of drupelets depends on weather conditions during pollination and fertilization. Studies of the phenology and yield dynamics of *R. chamaemorus* showed that this plant requires stable warm weather conditions in the flowering and fruiting periods. Cloudberry plants produce more fruit in wooded than in open habitats.

The plant spreads mainly by means of branched rhizomes, which are up to 10 m long and grow about 10–15 cm below the soil surface. New aboveground shoots develop from juvenile buds on the rhizomes. As a result of such vegetative propagation, an individual clone can cover an area of several square meters.

Sexual reproduction in this species is very slow and inefficient. Under experimental conditions, germinability varies from 0% to 31%. The drupelets have a thick endocarp, which can impede germination. Cloudberry “seeds” do not germinate unless the tough and bony endocarp is broken down or removed. Nicking of the seedcoat and endosperm is also necessary for in vitro germination (Warr et al. 1979, Thiem 2002). Therefore, scarification and stratification with the use of liquid nitrogen, sulphuric acid or NaOCl are usually needed for seed pregermination (Rantala 1976, Mian et al. 1995, Peacock & Hummer 1996). Gibberellic acid induces a significantly higher germination rate (Warr et al. 1979). Seedling growth is slow and the plant needs seven years to reach the flowering stage (Østgård 1964).

Decomposing fruit may be infected with *Botrytis cinerea* Pers. ex Fr., which externally looks like black sclerotia, as well as with *Fenicillium thomii* Maine (Taylor 1971). During early summer in Norway, some stromatic fungi – *Botrytis cinerea*, Cibora latioes, Rutstroernia chamaemori and Sclerotina tetraspora (Holst-Jensen & Schumacher 1994) – were also observed on cloudberry. Moreover, some larvae and parasites are commonly found feeding on its leaves (Taylor 1971, Weber 1995).

*R. chamaemorus* inhabits mainly peaty moors and bogs on mountains. Those habitats are generally acidic and poor in nutrients. The species occupies also a variety of moist tundra habitats, often growing profusely in patches of sphagnum moss. Individuals growing in different habitats express phenotypic variation. They have long shoots and large leaves at shady sites, but short shoots and small leaves in open areas. Mycorrhiza does not occur in *R. chamaemorus* in arctic Norway, Greenland and at British localities (Taylor 1971, Lohi 1974, Ågren 1989, Taylor 1990, Yudina 1993, Korpeleinen 1994).

This species is a boreal circumpolar plant (Fig. 2). It has a limited distribution on islands of the Arctic and occurs in Spitzbergen and Greenland. It is widespread
on three continents. In North America it extends from Alaska across Canada to Greenland and Labrador and south to New York. In Europe it is found mainly in Russia, Norway, Sweden, and Finland. Throughout the Scottish Highlands it is one of the most widespread mountain plants. It occurs at altitudes of up to 1400 m above sea level in Norway. In Central Europe, \( R. \text{chamaemorus} \) is rare: few localities of this species have been recorded in Germany, Poland, and in north-western Czech Republic. In Asia, the southern limit of its range extends eastwards through Russia, northern Manchuria and Mongolia to northern Japan (TAYLOR 1971, HULTEN & FRIES 1986, WEBER 1995).

![Fig. 2. Circumpolar distribution of \( R. \text{chamaemorus} \) L. (WEBER 1995; published by courtesy of Blackwell Verlag, Berlin)](image)

In Poland, cloudberry is a glacial relict under strict protection. It is scattered mainly in the northern part of the country: along the Baltic coast, in Pomerania and Masurian Lakeland. It occurs also at some isolated localities in the Sudeten Mountains in south-western Poland. At present it grows at about 20 sites, usually covering small areas (Fig. 3) (ZAJAC & ZAJAC 2001). This species is included in the list of rare and endangered plants in Poland (ŻUKOWSKI & JACKOWIAK 1995), the Polish Red Data Book of Plants (KRUSZELNICKI & FABISZEWSKI 2001) and the German
“Roten Liste” (WEBER 1995). *Rubus chamaemorus* is endangered mainly because of peatland drainage and peat exploitation. The distribution of cloudberry populations in Poland and Lithuania, as well as the ecology of this plant, were presented by GOSTYŃSKA-JAKUSZEWSKA & LEKAVIČIUS (1994). In Poland, cloudberry has been observed in bog pine woods in the association *Vaccinio uliginosi-Finetum*, and on peatbogs of the Atlantic type in the associations *Sphagnetum medii* and *Sphagnetum rubellii*. Environmental conditions and phytosociological descriptions of the peatbogs of East Pomerania and the reserve “Janiewickie Bagno”, which are some of the largest sites of *R. chamaemorus* in Poland, were presented by POLAKOWSKI (1962) and KRÓL (1968). Another area of occurrence of this species, the Slowinski National Park, where its plants also reached the fruiting stage, was described by CHRZANOWSKI (1992).

SECONDARY METABOLITES AND IMPORTANCE OF *RUBUS CHAMAEMORUS*

Orange or amber-coloured cloudberries are some of the best and most important available kinds of fruit. They are delicious, with sweet aromatic juice, and are picked for many uses. They are eaten fresh or frozen, stirred into Eskimo “ice cream”, and cooked into jams and pies. Cloudberries also serve as a material for making wines, liqueur, sweets, syrups and confitures. *R. chamaemorus* fruit and herbal tea made of it, are extremely rich in vitamin C, which is essential for human health (NORDNES & WERENSKIOLD 1951, ØSTGÅRD 1964, RYVARDEN 1993, HÄKKINEN et al. 1999b). Cloudberries retain vitamin C well if they are frozen or otherwise preserved immediately after picking. They contain benzoic acid, which is a natural preservative, and are a source of micro- and macronutrients: Fe, Cu, Mn, Zn, Mg, K, Ca, P (KOIVISTOINEN et al. 1974, HARJU & RONKAINEN 1984, KUHNLEIN et al. 1994). Seasonal variation in K content of different parts of *R. chamaemorus* growing in peat moss, was described by SÆBØ (1977). The essential oil extracted from the fruit contains 80 components (HONKANEN & PYYSALO 1976), including vanillin and its derivatives (PYYSALO & HONKANEN 1977). In the oil isolated from seeds (9.1–12.4%), triacylglycerols have been identified, and linoleic, α-linolenic, oleic and palmitic acid account for over 95% of all fatty acids (LAAKSO & VOUTILAINEN 1996, JOHANSSON et al. 1997). After supercritical carbon dioxide extraction, carotenes and tocopherols were found in the seed oil, beside major constituents of fatty acids (MANNINEN et al. 1997). A new chromatographic method was developed and applied to the analysis of cloudberry oil (MANNINEN & KALLIO 1997).

Flavonoids and phenolic acids are two large groups of phenolics, present in many kinds of edible fruit, including cloudberry. The study conducted with the aim to screen the major phenolic compounds in edible fruit of some species growing in the wild or cultivated in Finland, was initiated by TÖRRÖNEN et al. (1997). Freeze-dried fruit was subjected to extraction with methanol, and after hydrolysis the distribution of flavonol, ellagic acid and other phenolic acids was analysed by high-performance liquid chromatography (HPLC). In cloudberrries, the main phenolic compound was ellagic acid, but small amounts of flavonols and other acids were also detected. HÄKKINEN et al. (1998, 1999a, b) and TÖRRÖNEN (2000) identified the following substances: flavonol aglycons (quercetin, kaempferol, myricetin), ellagic
acid, hydroxycinnamic acids (p-coumaric, caffeic, ferulic) and p-hydroxybenzoic acids in various kinds of fruit from the families Rosaceae, Ericaceae, Grossulariaceae, Elaeagnaceae and Empetraceae. Cloudberries have a high ellagic acid content (e.g. 160 mg per 100 g seedless dry weight) and a small amount of other acids and flavonols (mainly quercetin) (HÄKKINEN et al. 1999a, b). After extraction and hydrolysis, the ellagic acid content of cloudberries determined by HPLC was found to be 60 mg per 100 g fresh weight (HÄKKINEN et al. 2000). It is interesting that certain kinds of fruit low in flavonols (cloudberry, arctic bramble, strawberry and raspberry) have a very high ellagic acid content. Thus *R. chamaemorus* fruit could play an important role in the dietary intake of ellagic acid, a potential anticarcinogen. Determination of a high level of ellagic acid in cloudberry leaves indicates that they could be used as a raw material for pharmaceutical purposes (KRAWCZYK et al. 2003). Cloudberries contain rubixanthin (3-hydroxy-β-carotene), used as a natural food-colouring compound (HARBORNE et al. 1999), and phytoestrogens (MAZUR et al. 2000). Phenolic compounds obtained from in vitro shoot culture and intact leaves by two-dimensional thin layer chromatography (2D-TLC) have also been compared (THIEM 1998). Some compounds found in *R. chamaemorus* are presented in Fig. 4.

Compounds obtained from fruit and leaves of *R. chamaemorus* have multiple biological properties beneficial for human health. Dietary flavonoids and phenolic acids, especially ellagic acid, are known to be antioxidants, having antimutagenic, anticanncinogenic, hepatoprotective, and antimicrobial properties (e.g. TANAKA et al. 1993, KNEKT et al. 1997, CHUNG et al. 1998, RAUHA et al. 2000, PUUPPONEN-PIMIÄ et al. 2001). Ellagic acid, a product of degradation of certain plant tannins, is also an endogenous inhibitor of insect growth and deters insects feeding on certain plants (MAAS & GALLETTA 1991). Additionally, ellagic acid has been recently allowed for use as a food additive in Japan, functioning as an antioxidant (AMAKURA et al. 2000). Flavonoids and phenolic compounds contained in cloudberry and in some other plants affect calcium transport in animal cells (RAUHA et al. 1999). The performed tests may help to explain health effects of the dietary usage of these plants.

Because of the economic importance of cloudberry in northern Scandinavia, studies of this plant arouse broad interest (KAURIN et al. 1981, 1982, KORTESHAJU 1995). Fruit sold there is picked on native peatland, and its yield is 20 to 50 kg/ha. Cultivation methods for commercial cloudberry production have been developed in Norway, Finland and North America (e.g. MÄKINEN & OIKARINEN 1974, LID 1985, KARDELL 1986, RAPP 1991, KULISHKINA & KOSITSYN 1995, WEBER 1995). This fruit, used as a raw material for industry, is of economical importance in Scandinavia, where the crop of this valued fruit is strictly controlled and legally regulated. Fruit and leaves, also rich in vitamin C and tannins, were an important remedy against scurvy and diarrhoea in traditional medicine.

**IN VITRO CULTURE STUDIES**

Since changes in the environment lead to disappearance of habitats of *Rubus chamaemorus* and its the vegetative propagation capacity is low, it has become necessary to use biotechnological methods for protection and preservation of this species. The most useful method of vegetative propagation under in vitro conditions,
Fig. 4. Some compounds found in *Rubus chamaemorus* L.
which preserves plant genotype, is multiplication from existing meristems (BAJAJ et al. 1988).

A method for micropropagation of *R. chamaemorus* from shoot tips has been developed by initiation of axillary shoots (THIEM 2001a). The highest multiplication rate of cloudberry shoots (Fig. 5) was achieved on MS medium (MURASHIGE & SKOOG 1962) with a very low pH of 4.0, and containing 6-benzyladenine (BA) and indole-3-butyric acid (IBA). Difficulties in root induction on the regenerated shoots of the genus *Rubus*, reported by several authors (ANDERSON 1980, SOBCZYKIEWICZ 1980, HOEPFNER & NESTBY 1991), induced multidirectional experiments with cloudberry rooting in vitro and ex vitro. In vitro-derived plantlets were transferred into pots and, after acclimatization, planted in the garden.

Callus was obtained from fragments of seedlings. Only young tissues proliferated callus on ½ MS medium supplemented with auxins: 2,4-dichlorophenoxyacetic acid (2,4-D) or α-naphthaleneacetic acid (NAA) and small amounts of cytokinin. Induction of slow-growing, undifferentiated callus and its growth, were observed on ½ MS medium containing NAA and kinetin, after many studies with several plant growth regulators (THIEM, unpubl. results).

Two biotechnological methods for conservation of *R. chamaemorus* shoot cultures have been developed by the medium-cold storage and encapsulation of shoot buds with a high conversion ability (THIEM 2002). The shoot cultures stored at 4°C and low light intensity for 12 months without an intervening subculture survived with 90% viability. Axillary buds obtained from in vitro shoot culture of cloudberry were encapsulated in calcium alginate hydrogel. Encapsulated buds stored at a low temperature in the dark survived for up to 3 months without loss in viability. The fidelity of the regenerated plantlets was evaluated by a phytochemical analysis of some phenolic compounds and morphological observations. The plantlet ploidy level was controlled and determined by flow cytometric methods (THIEM & ŚLIWIŃSKA 2003). Preliminary trials in the botanical garden showed that in vitro-derived plantlets of *R. chamaemorus* can be used for conservation ex situ. This confirms that in vitro culture techniques could be used for conservation of rare and endangered species.

An earlier in vitro study of *R. chamaemorus* was conducted by NAESS et al. (1993). They used shoot organogenesis from leaves as a technique for mixoploid production following interspecific hybridization.

The application of in vitro clonal propagation of *R. chamaemorus* offers an alternative method for production of some numbers of uniform plants for introduction to botanical gardens and for in vitro germplasm storage of this endangered species (THIEM 2001b, 2002).

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Fig. 1. *Rubus chamaemorus* L. in the Słowiński National Park (phot. by J. HERBICH)
Fig. 3. Distribution of *Rubus chamaemorus* L. in Poland (ZAJAC & ZAJAC 2001; published by courtesy of Institute of Botany, Jagiellonian University, Kraków)
Fig. 5. In vitro shoot cultures of *Rubus chamaemorus* (phot. by B. THEM)