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**EVALUATING GERMPLASM OF REED CANARY GRASS,  
*Phalaris arundinacea* L.**

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ACADEMIC DISSERTATION

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## ABSTRACT

Reed canary grass, *Phalaris arundinacea* L., is native to northern temperate regions and is widely distributed throughout Europe, Asia, America and Africa. It has a long history as a feed crop, but it is also a potential crop for bioenergy and paper pulp production under northern conditions. Furthermore, it has environmental value for filtration and evaporation of runoff water and in erosion control. Reed canary grass is an attractive field crop because of its high biomass yield and persistence under wet and dry conditions and on many soil types. However, its high alkaloid content has been harmful to ruminants and therefore breeding, mainly in North America, has targeted increased forage yield and better palatability. In the 1990s agro-industrial usage of crops was introduced in Nordic countries. Delayed harvesting, a new method, was developed for non-food production of reed canary grass. Breeding reed canary grass for non-food production started in Finland and in Sweden with specific aims to produce high biomass yield with increased stem to leaf mass compared with existing forage cultivars. Wild populations have been used in breeding in order to benefit from local adaptation and new variation in traits important in non-food production. Breeding reed canary grass is characterized by its outcrossing, perennial and polyploid nature and possibilities for vegetative propagation.

The main objective of this study was to describe variation in agronomic traits in reed canary grass germplasm. Another aim was to identify geographic variation in agronomic traits among wild germplasm accessions and evaluate the potential of current germplasm for non-food and seed production and for breeding. This study included fifty-three wild populations of Finnish origin from 60-66°N and eight cultivars and fourteen breeding lines mainly from central Europe and North America (ca. 10-15°N south of Finland). In total, 23 agronomic traits were evaluated between 1994 and 1998 in field trials at Jokioinen in Finland. Ideal plant types for non-food and seed production were described: a non-food ideotype should be tall, strong, have an unbranched stem and few, small leaves. A plant stand should contain many stems, be winter hardy, have low mineral content and be resistant to pests and diseases. An ideotype for seed production should have high seed yield accompanied by even seed ripening, low seed shattering, good germination and high thousand seed weight.

The present study revealed that both the elite and wild populations varied for each trait. Wild populations also exhibited geographic distribution, which should be exploited in locating particular traits of interest for breeding and targeting new germplasm collections. The best populations for each end-use were indicated by an index designed around various traits. Forage cultivars and breeding lines had better biomass and seed yield traits than wild populations. Although wild germplasm was inferior to elite material as a whole, some possessed a desirable combination of traits for different end-uses and could be usefully incorporated into breeding programmes. For instance, wild populations had better winter-hardiness, higher straw or leaf proportion and unbranched stems. On the basis of the results it is concluded that this material provides a realistic coverage of reed canary grass genetic resources. Thus, this study may form the basis of a full-fledged breeding programme for improved cultivars. At this point promising local populations identified in this study serve as well-described candidates for breeding. It is possible to select populations *per se* for cultivar trials, pick components for single crosses or form a synthetic cultivar. As material was tested in one location, the first selection should be mild, discarding only the poorest performers. Replicated trials of the best populations should then be carried out within a tentative cultivation region to gauge stability.

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which in the text are referred to by their Roman numerals.

- I Sahramaa, M. and Jauhiainen, L. 2003. Characterization of development and stem elongation of reed canary grass under northern conditions. *Industrial Crops and Products* 18: 155-169.
- II Sahramaa, M., Ihamäki, H. and Jauhiainen, L. 2003. Variation in biomass related variables of reed canary grass. *Agricultural and Food Science in Finland* 12: 213-225.
- III Sahramaa, M., Hömmö, L. and Jauhiainen, L. 2004. Variation in seed production traits of reed canarygrass germplasm. *Crop Science* 44: 988-996.
- IV Sahramaa, M. and Hömmö, L. 2000. Seed production characters and germination performance of reed canary grass in Finland. *Agricultural and Food Science in Finland* 9: 239-251.
- V Sahramaa, M. 2003. Evaluation of reed canary grass for different end-uses and in breeding. *Agricultural and Food Science in Finland* 12: 227-241.

## LIST OF ABBREVIATIONS

DAA	days after anthesis
dd °C	degree days (accumulation of temperature above + 5 °C)
DM	dry matter
F <sub>ST</sub>	divergence in marker genes
G x E	genotype by environment interaction
LAI	leaf area index
Mtoe	energy unit corresponding a million equivalent oil ton
RCG	reed canary grass, <i>Phalaris arundinacea</i> L.
TWh	terawatt hour
Q <sub>ST</sub>	divergence in quantitative traits

## 1. INTRODUCTION

### 1.1. Taxonomy and origin

Reed canary grass (*Phalaris arundinacea* L.; hereafter RCG) was described by Carl von Linné in 1753. It belongs to the grass family *Poaceae*, genus *Phalaris* and tribe *Phalarideae*. Genus *Phalaris* has a complicated taxonomic and nomenclatural history. It comprises 22 species, mostly from temperate regions (Table 1). The basic chromosome number of RCG is seven and the regular formation of 14 bivalents at meiosis suggests it is an allopolyploid (Ambastha 1956, Starling 1961). In genetic studies it is considered a diploid. However, two chromosome numbers have been found, one tetraploid ( $2n=28$ ) and the other hexaploid ( $2n=42$ ) (McWilliam and Neal-Smith 1962). Most RCG in Europe and North America is tetraploid. The tetraploid state has also been verified from the wild growing material in Finland (Uotila and Pellinen 1982). Tetraploids are more widespread and cold hardy than hexaploids, which are adapted to warmer environments (McWilliam and Neal-Smith 1962). Hexaploids tend to be more productive and have no winter dormancy compared with tetraploids (McWilliam and Neal-Smith 1962). In Finland, RCG is the only native *Phalaris* species. It is common up to Finnish Lapland (Hämet-Ahti et al. 1998). It grows naturally in wet habitats; along streams, lake margins, springs and meadows. Another *Phalaris* species growing in Finland is *Phalaris canariensis* L., which is a casual alien, found after 1950 (Hämet-Ahti et al., 1998). In contrast to RCG, *P. canariensis* is annual with a chromosome number  $2n=12$ .

Species of the genus *Phalaris* are present in all continents except Antarctica. *Phalaris* has two main centres of origin: the Mediterranean and southwestern USA (Baldini 1995). The Mediterranean annuals and New World annuals are self-pollinating, whereas the perennial species are largely self-incompatible (Carlson et al. 1996). *Phalaris aquatica* L., which is called hardinggrass or simply phalaris, is taxonomically most closely related to RCG, both of them being perennials (McWilliam and Neal-Smith 1962). Like many other grasses, RCG has a Mediterranean origin (Baldini 1995). It is the only species that links two *Phalaris* centres of origin with its circumboreal distribution. RCG is native in the northern temperate zone and widely distributed throughout Europe, temperate Asia, America and Africa. However, it is disputed whether it is native in North America or was introduced from Europe. North American populations may be mixtures of native populations and European cultivars, because RCG appears to have been present in northwestern USA before Euro-American settlement (Merigliano and Lesica 1998). The European RCG is considered more aggressive in growth than the American populations (Merigliano and Lesica 1998).

Table 1. Synopsis of genus *Phalaris* L.

Species name	Common name(s)	Finnish name	annual/ perennial	Chromosome number 2n	General distribution	Usage
1 <i>Phalaris amethystina</i> Trin.			annual	unknown	Chile, Juan Fernandez Islands	
2 <i>Phalaris angusta</i> Nees ex Trin.	Timothy canarygrass	kapeätäähkähelpi	annual	14	North and South America, adventive in South Africa and Europe, introduced in Australia	forage
3 <i>Phalaris appendiculata</i> Schult.			annual	unknown	Middle East, Africa	
4 <i>Phalaris aquatica</i> L. Cent ( <i>Phalaris tuberosa</i> L.) ( <i>Phalaris nodosa</i> L.) ( <i>Phalaris bulbosa</i> L.)	Phalaris, Hardingsgrass, Koleagrass, Toowoomba canarygrass, Bulbous canarygrass,		annual perennial	28	Australia, Mediterranean region, introduced to Northern Europe, Northern and Southern America, Africa, New Zealand	forage (mainly pasture)
5 <i>Phalaris arundinacea</i> L.	Reed canary grass	ruokohelpi	perennial	28, 42	Tetraploid: Europe (native to Finland), North and South America, temperate Asia, South Africa; Hexaploid: Australia, New Zealand native in the northern temperate zone	forage, bioenergy, paper, environmental usage ornamental
var. <i>picta</i> L.	Ribbon grass Gardener's garters	viiruhelpi	perennial	14,28,42		
6 <i>Phalaris brachystachys</i> Link in Schrad.	Shortspike canarygrass		annual	12	Mediterranean region, adventive in North Europe, introduced in North America and in Australia	weed
7 <i>Phalaris caesia</i> Nees			perennial	42	Europe, Africa, Middle East	
8 <i>Phalaris californica</i> Hook.	California canarygrass		perennial	28	USA (California, Oregon), Mediterranean region, Australia	
9 <i>Phalaris canariensis</i> L.	Canarygrass, Annual canarygrass	kanarianhelpi	annual	12	Mediterranean region, USA, Canary Isles, Argentina, Australia, Morocco, Canada	bird-food
10 <i>Phalaris caroliniana</i> Walt.	Carolina/Carolina canarygrass	lämmenhelpi	annual	14	USA, Puerto Rico, Mexico, adventive in Europe, introduced in Australia	weed, forage
11 <i>Phalaris coeruleascens</i> Desf.	Blue canary-grass, Sunolgrass		perennial	14,28,42	Mediterranean region, adventive in North Europe and in South America, introduced in Australia	weed
12 <i>Phalaris daviesii</i> S.T.Blake			perennial	56	Australia, Africa, South America	forage
13 <i>Phalaris elongata</i> Braun-Blanq.			perennial	28	Western Mediterranean, Northern Africa	
14 <i>Phalaris lemmonii</i> Vasey	Lemmon's canarygrass		annual	14	USA (California), introduced in Australia	

15	<i>Phalaris lindigii</i> Baldini			perennial	unknown	Colombia, Ecuador
16	<i>Phalaris maderensis</i> Menezes			annual	28	Isle of Madeira, Porto Santo and the Canary Islands
17	<i>Phalaris minor</i> Retz.	Littleseed canarygrass, Lesser canary-grass	pikkuhelpi	annual	28	Mediterranean, adventitious in Africa, introduced to North and South America, Australia and Far East, occasionally northern Europe and Finland
18	<i>Phalaris paradoxa</i> L.	Awned canarygrass, Hood canarygrass	rikkahelpi	annual	14	Mediterranean region, adventive in North Europe, introduced in North and South America and Australia
19	<i>Phalaris peruviana</i> H. Scholz & Gutte			perennial	unknown	Peru, Colombia, Mexico
20	<i>Phalaris platensis</i> Henrard ex Wacht.			annual	14	South America (Uruguay, Brazil, Argentina), adventive in North Europe
21	<i>Phalaris rotgesii</i> Husn.			perennial	14	France, Corsica, Italy, Sardinia
22	<i>Phalaris truncata</i> Cuss. ex Bert.			perennial	12	Mediterranean region, adventive in North Europe, USA

References: Ambastha 1956, Baldini 1995, Carlson et al. 1996, McWilliam and Neal-Smith 1962, Matus and Hucl 1999, Matus-Càdiz and Hucl

## 1.2. Description

RCG is a vigorous, leafy, erect and long-lived grass (Figure 1). Stems are usually 1.5- 2 m high with many leaves and a panicle. The panicle is dense, usually bluish-green or reddish. Spikelets are situated at the top of the panicle branches. The panicle is closed before and after anthesis and open during anthesis. Flowering of this cross-pollinating species takes place in mid-summer, usually in the mornings. As with most of the other temperate perennial grasses, RCG has a dual photoperiodic induction requirement for flowering. It is classified as a short-long-day plant, which has both the primary and secondary induction requirement. Primary induction requires exposure to short days for 12 to 18 weeks at temperatures ranging from 6 to 15°C (vernalization), while secondary induction requires long days and is enhanced with moderately high temperatures (Heide 1994). RCG panicles tend to shatter the seeds, which takes place in two-stages: seed becomes free as a result of disarticulation of the rachilla about 12 days after anthesis, which is followed by release of seeds from the glumes (Bonin and Goplen, 1963). Seeds are small; thousand seed weight averages 0.9 g. The mature seed is dark grey or brown and about 3 mm long. In addition to sexual reproduction by seed, RCG can reproduce vegetatively. Clonal reproduction takes place by rhizome growth or even by plant parts. It produces roots and shoots from the nodes of culms (Marten and Heath 1973). Parts of panicles with intact seeds can spread widely through water, which is the usual element in habitats of RCG. It has an expansive underground rhizomatous root system from which single stems arise (Evans and Ely 1941).

## 1.3. History and future potential

RCG has a long history as a feed crop. It was reported from Sweden as early as 1749 as a palatable forage grass (Alway 1931). In Norway, it was studied in 1870 (Østrem 1987) and in Finland, the first trials were documented in 1886 in Herniäinen, South Häme (von Essen 1913). It was first reported in England by 1824 and in Germany in about 1854 (Alway 1931). The first North-American trials were reported in the 1830s (Merigliano and Lesica 1998). It is used for pasture, silage and hay at temperate latitudes. It is persistent under grazing and probably best suited for that purpose (Carlson et al. 1996). In Australia RCG is mainly used for grazing sheep, although its near relative, *P. aquatica* is a more common species there covering an area of 1.5 million hectares (Carlson et al. 1996). In addition to surviving the hot and dry summers of Australia, RCG is also waterlogging tolerant. It did not suffer from occasional covering of water in a Finnish study (Lindh et al. 2002). As compared with other forage grasses, it has shown high DM yield, adaptation to many soil types, but lower feeding value (Carlson et al. 1996). High alkaloid content of RCG has



Figure 1. Reed canary grass (*Phalaris arundinacea* L.) (Drawing: Helena Ihmäki)

caused poor weight gains and incidence of diarrhoea in cattle (Marten et al. 1976). Alkaloid concentration is also negatively correlated with palatability. Nine alkaloids have been found in RCG including gramine, hordenine, four tryptamine derivatives and three  $\beta$ -carbolines (Østrem 1987). Gramine type alkaloids have been shown to be least harmful to the ruminants and digestibility has been improved through breeding low-gramine cultivars.

Despite its relatively long history as a feed crop, RCG is a moderately new non-food crop. In Nordic countries the agro-industrial usage of crops for paper and bioenergy was initiated at the end of the 1980s. The driving force towards non-food cultivation was an increasing area of set-aside land, which was estimated to be 0.5 to 1 million hectares in Finland up to 2000. Currently fields not used for food or forage cover 530 000 hectares (Anon. 2003). Non-food production offers new income possibilities for farmers and keeps fields in cultivation, which can then be easily brought back into food production. A new harvest method, delayed harvesting, was developed for non-food production of RCG. In this system the grass stands are left over winter and harvested in the spring before appearance of new green shoots (Landström et al. 1996). This was considered the most successful method under northern growing conditions (Landström et al. 1996, Pahkala 1997). The biomass yield was highest at spring harvest in the long term (Pahkala and Pihala 2000) and the quality of spring harvested biomass was better for non-food because of a lower mineral content (Burvall 1997). The moisture content of the dead grass is about 10 to 15% and no artificial drying of biomass is needed. Two basic methods for harvest have been used in Finland: baling (Suokannas and Serenius 2000) and loose harvest (Lindh et al. 2000). Under Nordic conditions RCG has yielded 6 to 8 t DM ha<sup>-1</sup> (Landström et al. 1996, Saijonkari-Pahkala 2001). In addition to above-ground biomass, below-ground biomass is about half of the total plant biomass (Kätterer and Andréén 1999).

During the beginning of the 1990s the Finnish paper industry lacked domestic short fibre, and its substitution with herbaceous crops was the primary non-food interest. In fine paper production both long and short fibres are needed: long fibres from softwood conifers like spruce (*Picea abies* (L.) H. Karst.) and Scots pine (*Pinus sylvestris* L.) and short fibres from hardwood birch (*Betula* L.). Among 17 herbaceous species studied, RCG was the most promising for fine paper production having high biomass yield and being of good pulping quality (Saijonkari-Pahkala 2001). Pulp yield per hectare of RCG was almost double that of birch and pilot-scale tests revealed that its pulp could replace birch pulp without adversely affecting paper properties (Paavilainen and Tulppala 1996). Economic evaluation indicated that fine paper production from RCG could be profitable business; the internal rate of return was almost identical to that for wood (Paavilainen and Tulppala 1996). In that case the reed line should be integrated into a sulphate pulp mill. However, currently RCG is not used for paper-making in Finland, although both the technique and

economics would allow it. The main reason for that has been low interest of the pulping industry to invest in development of reed lines, and the ease of getting birch for pulping.

Another non-food use of field crops, cultivation for energy production, was also initiated in the 1990s. Environmental problems caused by burning fossil fuels, enhancing the greenhouse effect in particular, stimulated interest in use of renewable resources. Currently European energy politics aims to produce 90 Mtoe of bioenergy up to 2010, of which half is planned to come from field-based energy crops. This will need 10 million hectares of arable land of the EU's total of 77 Mha (Anon. 2001). In Finland RCG represents a potential energy crop, with willow (*Salix* L.), turnip rape (*Brassica rapa* L.ssp. *oleifera* DC.) and also straw as a by-product from cereal production. The possible fuel products are solid fuels, motor fuels, pyrolysis oil and biogas. In 2003 the target for energy crops in Finland was set to be 2.5 TWh to 2010, of which 2.03 TWh would come from RCG (Leinonen et al. 2003). That corresponds to 75 000 hectares arable land including cut-over peat production areas, which is about 1 000-2 000 hectares per year. At the present time RCG is cultivated on 2 700 hectares in Finland (Anon.2003) and is used both for bioenergy and for forage. Results from Finnish studies have shown that 10% of RCG can be used in a fuel mixture together with peat or wood chips without modifications becoming necessary to the fuel handling equipment of the power plants (Flyktman 2000). Furthermore, dozens of existing heating plants are suitable for burning agrobiomass (Palonen and Laine 1998). The largest private energy company in Finland, Pohjolan Voima Oy, has started a project in Ostrobothnia with four power plants with the goal of launching cultivation of RCG for large-scale energy production. The aim is to cultivate RCG on approximately 4 000 hectares before the end of 2005. Finnish peat enterprise Vapo Oy Energy has already established 2 100 hectares of RCG on its own peat production areas. Market price of RCG, 34 € per tonne, is linked with that of by peat, which is the most similar energy source. The production costs are 65-68 € per tonne including 30 km transport to the power plant (Klemola et al. 2000, Lindh et al. 2000). However, bales need to be chaffed before use, which increases the preparation cost. Including agricultural subsidies, which were 270 – 532 € per hectare in 2002 depending on the subsidy area and form, cultivation of RCG for non-food is profitable in Finland (Pahkala et al. 2002).

In addition to paper pulp and bioenergy, RCG is also of environmental value. It has been demonstrated to be a good catch crop for nutrients (Partala et al. 2001) and could be used for example in buffer zones to prevent nutrient leaching. Runoff waters from peat production areas have been filtrated and evaporated by RCG (Lindh and Paappanen 1999, Lindh et al. 2002, Puuronen et al. 1998). RCG could also control nutrient losses from livestock wastes (Studdy et al. 1995) and it is valued as a soil binder for erosion control because of its large rhizomatous root system (Bernard and Lauve 1995). Although RCG

has many uses, in the USA it is often considered an invasive weed, which limits the growth of other species (Morrison and Molofsky 1998, Miller and Zedler 2003). In some regions or habitats it may displace desirable native vegetation if not properly managed. However, RCG is native to Finland and grows over the entire country. Hence, its invasive character seems not to be a threat in Finland; it forms natural stands of only a couple of square meters, mostly along riversides and roadsides.

#### **1.4. Breeding advances and research**

Relatively few cultivars of RCG have been developed. Most breeding work has been for forage in the United States and Canada. Breeding has aimed at improved seed retention and increased seed and forage yield for improved palatability. Some forage cultivars released include ‘Auburn’ (Alabama, year of release 1952), ‘Castor’ (Canada, 1972), ‘Flare’ (USA, 1974), ‘Frontier’ (Canada, 1959), ‘Grove’ (Canada, 1970), ‘Ioreed’ (USA, 1946), ‘Palaton’ (USA, 1985), ‘Rise’ (USA, 1965), ‘Rival’ (Canada, 1985), ‘Vantage’ (USA, 1972) and ‘Venture’ (USA, 1985) (Carlson et al.1996). Cultivar ‘Superior’ (USA, 1930) differs from others being hexaploid and therefore, having larger seeds (Carlson et al.1996). In Norway, a national forage breeding programme started in 1978 at Løken Research Station. Breeding has aimed at developing a cultivar adapted to Norwegian climatic conditions with better forage quality. As a result ‘Lara’ was released in 1992 (Marum and Solberg 1993). It is the only forage cultivar developed in the Nordic countries.

Breeding RCG for industrial uses started in 1989 at Svalof Weibull in Sweden (Lindvall 1997), and in 1993 at MTT Agrifood Research Finland. Breeding aims were specified for industrial use of the crop: an ideotype energy or fibre plant was required to be tall, have a robust stem and preferably no leaves (Lindvall 1997). In Sweden local material and European genebank accessions were crossed through open pollination to establish breeding populations. Material was evaluated for yield potential, quality and adaptation to different climatic conditions (Lindvall 1997). Breeding continues and the first non-food cultivar ‘Bamse’ has recently been released. It showed a 20% higher biomass yield than cultivar ‘Palaton’. In Finland MTT evaluated agronomic traits of 75 populations including wild and elite RCG, and Boreal Plant Breeding Ltd. will continue the breeding of the most promising populations. On the basis of that work, the first domestic non-food cultivar should be released around 2010.

To develop a suitable forage crop, high alkaloid content has been decreased and digestibility improved through breeding of low-gramine cultivars like ‘Palaton’ and ‘Venture’. A genetic model of alkaloids was presented by Marum et al. (1979b). They found that gramine-type alkaloids were synthesized only in the double recessive genotype.

As RCG contains alkaloids primarily from one group, double recessive enables plant breeders to select directly for gramine type without progeny testing. In genepools of RCG, including local Norwegian material, gramine occurred with the highest frequency of the alkaloid types studied (Marum et al. 1979b, Østrem 1987). Variation in alkaloid concentration and its heritability was sufficiently high to expect response to selection in breeding (Østrem 1987). Another advance in forage breeding was development of RCG somaclones in the Hungarian breeding programme. Somaclones were initiated from callus cultures of young inflorescence for the generation of new genotypes with improved palatability and digestibility (Mester et al. 1998).

Interspecific hybridization has been used in RCG breeding. It has also been shown that RCG races can be hybridized with the Mediterranean tetraploid *P. aquatica* (Starling 1961, McWilliam and Neal-Smith 1962). The sterile hybrid is called Ronphagrass (McWilliam and Neal-Smith 1962). The possibilities for transfer of the intact rachilla trait of *P. aquatica* to RCG (Carlson et al. 1996) and recombination of the palatability of *P. aquatica* and the winter-hardiness of RCG (Starling 1961) have both been of interest to plant breeders. It might also be of interest to hybridize two chromosome races of RCG in order to benefit from the higher yield potential of the hexaploid race. However, resulting hybrids have shown to be sterile (McWilliam and Neal-Smith 1962).

### **1.5. Breeding methods**

The most important characteristics affecting breeding of RCG are that it is an outcrossing, perennial, polyploid species, which can be vegetatively propagated. As a result of outcrossing improved cultivars are heterozygous populations and due to it being perennial, it can be maintained for several years. Although an allopolyploid, it is a functional diploid. Most existing cultivars are multiple-clone synthetics (Kalton et al. 1989ab), where several parents are randomly mated so that all possible matings have equal probability of occurring (Busbice 1969). Parents are usually tested for their combining ability through top cross, single-cross, or more generally, polycross methods (Briggs and Knowles 1967). Superior parents are selected to establish a synthetic cultivar and seed is increased in successive generations without selection. Inbreeding in a synthetic cultivar increases as the number of parents decreases. Inbreeding leads to a decrease in vigour and therefore, a productive cultivar has to originate from a sufficient number of parents in order to avoid inbreeding depression in subsequent generations. At least four parents should be used and the optimum number would be closer to ten (Briggs and Knowles 1967). In practice, grasses are generally multiplied twice and are thus considerably removed from the first synthetic generation, Syn<sub>1</sub> (Simmonds 1979). Syn<sub>1</sub> seed is frequently formed by composing equal

quantities of seed from parents and the breeder should be able to reconstitute the cultivar at any time.

Phenotypic and mass selection are the most common recurrent selection methods for forage breeding. Individual plants with desirable traits are bulked to produce the following generation (Casler et al. 1996). Phenotypic selection differs from mass selection in that pollination of selected individuals is controlled (Casler et al. 1996). Promising parental lines could also be crossed in diallel without progeny testing and selection redone among the segregating progeny. The development of inbred lines and exploitation of hybrid vigour (heterosis) has rarely been used in RCG breeding, although it has been suggested (Knowles 1986). As hybrid vigour is maximized only in the first filial generation, RCG could benefit because of its strong rhizomatous spreading ability and ease of vegetative propagation. Although commercial production of hybrid cultivars in RCG is not economically attractive at present, machinery for planting vegetative plant parts has been developed for use with other perennial grasses and may warrant further testing with this species (Marum et al. 1979a). Cytoplasmic male sterility could open possibilities to product hybrids on a commercial scale as with alfalfa (*Medicago sativa* L., Davis and Greenblatt 1967) and ryegrasses (*Lolium* spp., Wit 1974). RCG is a highly self-sterile species and only small amounts of selfed seed are expected under conditions of hybrid seed production (Marum et al. 1979a). Self incompatibility genes identified from a related species, *P. coerulescens* Desf. (Li et al. 1997), could also be of benefit in RCG breeding in the future.

### **1.6. New diversity from wild populations**

Genetic resources of a species include its wild relatives, landraces and primitive cultivars as well as advanced breeding lines and modern cultivars (Hoyt 1988). These groups represent the species genepool containing all its genetic variation. Genetic resources are important raw material for plant breeders because adequate genetic variation is needed for breeding new cultivars. The genetic base of modern cultivars has narrowed as a result of long-term breeding work, which is most clearly seen in modern cultivars of cereals including barley (*Hordeum vulgare* L.). This reduction of genetic diversity is termed genetic erosion (Hawkes 1990). Genetically uniform cultivars tend to be vulnerable to pests and diseases and less tolerant of environmental changes. Landraces and wild populations may contribute valuable traits and genes for breeding. The genetic diversity within a crop genepool is moreover of intrinsic value as a cultural heritage, which needs to be maintained *ex situ* and *in situ* when possible, for current and future needs.

Breeding cool-season forage grasses began around 1890 both in Europe and the USA (Casler et al. 1996). Although forage breeding has now been done for over a hundred years, exploitation of variation from local populations is still the mainstay in breeding (Casler et al. 1996). The main input of new diversity for major temperate forage grasses, *Bromus* spp., *Dactylis* spp., *Festuca* spp., *Lolium* spp. and *Poa* spp., has come from wild populations and from hybridizations (Smith 1995). For example a ryegrass collection in Switzerland contributed increased forage yield and quality for hybrids of perennial (*Lolium perenne* L.) x Italian ryegrass (*Lolium multiflorum* Lam.) (Jones and Humphreys 1993). In North America wild populations of perennial ryegrass were evaluated in order to estimate the variation and potential use for future germplasm exploration (Casler 1995). Genetic resources of RCG exist in all temperate regions of the world. Availability of locally adapted populations is important especially in marginal northern conditions. In RCG breeding programmes local populations have been used together with foreign material in Norway, Sweden and Finland. Breeders have searched for variation in traits important in forage or non-food production and local adaptation.

## 2. AIMS OF THE STUDY

In Finland, successful non-food or forage production of RCG requires breeding domestic cultivars adapted to northern growing conditions. Economic production requires effective seed production. The first step of the breeding programme is to evaluate a broad collection of basic material in order to establish the extent of variation in agronomically important traits. Exploitation of local wild populations in breeding improves adaptation of new cultivars to our marginal growing conditions. Knowledge of the geographic distribution of diversity is helpful in locating particular traits of interest for breeding. Future plant breeding will benefit from a better understanding of the intraspecific diversity in RCG.

In addition to breeding prospects, evaluation of RCG germplasm demonstrates the diversity of the species in Finland. Information on geographic variation will serve in germplasm conservation, when comprehensiveness of diversity of the Finnish RCG collections in the genebank is estimated and new germplasm collections are targeted. Novel end-uses of RCG in non-food production (bioenergy, paper pulp) and development of new cultivation methods (delayed harvest) require research related to agronomic traits.

There are many different dimensions to genetic variation, from the molecular level to major morphological traits detectable with the naked eye, which represent the first step in description and classification of germplasm. This study focused on describing variation in morphological, phenological and growth related traits of RCG. It is the basis of the breeding programme.

The aims of this study were:

- 1) To describe variation in agronomic traits in RCG germplasm (I, II, III)
- 2) To identify the possible geographic variation in agronomic traits in wild germplasm (I, II, III, V)
- 3) To evaluate the potential value of present germplasm for different end-uses and in breeding (IV, V)
- 4) To understand the associations among agronomic traits (II, III)

### **3. MATERIALS AND METHODS**

#### **3.1. Plant material and traits**

Seventy-five introductions of RCG (I, figure 1; V, table 1), representing collections from Europe, Russia and North America, were established in a trial at MTT Agrifood Research Finland in Jokioinen (60°49'N), in July 1994. Fifty-three introductions were wild populations gathered in 1993 from various regions of Finland between 60°N-66°N, and twenty-two were cultivars and breeding lines. Seeds were grown in pots in a greenhouse and seedlings were planted in the field. The material was grown in a randomized complete block design with four replications. Field experiments were carried out in 1994-1998. In another experiment seed production traits of cultivars Palaton and Venture were studied between 1995 and 1998 at Jokioinen. In total 23 agronomic traits were evaluated in this study, which are listed in Table 2. Details of the origin of plant material and traits studied are given in the respective papers (I-V).

#### **3.2. Statistical methods**

Statistical analyses used in these studies are listed in Table 2 and described in more detail in the respective papers (I-V). Analyses of repeated measurements and/or traditional ANOVA for a randomized complete block design were used. Furthermore, stem elongation was analysed using logistic growth curves and leaf area index (LAI) using second degree polynomials. The genotype by environmental interaction for seed production traits was measured plotting variables against environmental variables (III). Correlations between two variables were measured by using Pearson correlation coefficients or Spearman's rank-order correlation coefficients. Relationships among 12 variables were analysed using factor analyses (II). Traits for non-food (NF), forage (F) and seed production (SP) indices were analysed using ANOVA models for randomized complete block designs (V). Populations were ranked according to estimated means for each trait.

Table 2. Agronomic traits of reed canary grass evaluated in this study

No	Trait	Unit	Year	Paper	Transformation	Statistical analyses
1	Biomass yield	kg DM ha <sup>-1</sup>	1995-1998	II, V	log	RM†
2	Proportion of straw	%	1996	II, V	-	ANOVA
3	Proportion of leaves and leaf sheaths	%	1996	II, V	-	ANOVA
4	Proportion of nodes	%	1996	II, V	-	ANOVA
5	Proportion of shoots	%	1996	II, V	arsin sqrt	ANOVA
6	Shoot number	per stem	1996	II	-	ANOVA
7	Node number	per stem	1995-1996	II	-	RM
8	Panicle number	m <sup>-2</sup>	1995-1997	II, III, V	log	RM
9	Leaf area index	per surface	1995-1996	II, V	-	RM, quadratic polynomial
10	Emergence of flag leaf	dd	1995-1996	I	-	RM
11	Inflorescence visible	dd	1995-1996	I	-	RM
12	Emergence of inflorescence	dd	1995-1997	I	-	RM
13	Beginning of anthesis	dd	1995-1997	I	-	RM
14	Completed anthesis	dd	1995-1997	I	-	RM
15	Seed ripening	dd	1995-1997	I, II, V	-	RM
16	Plant height	cm	1994-1997	1, II, V	-	RM, logistic growth curves
17	Overwintering	%	1995-1998	II, V	arsin sqrt	RM
18	Seed yield	g panicle <sup>-1</sup>	1995	III, IV, V	sqrt	RM, ANOVA
19	Seed shattering	%	1995	III, V	arsin sqrt	RM
20	Thousand seed weight	g	1995	III, IV, V	-	RM, ANOVA
21	Seed germination	%	1995	III, IV, V	arsin sqrt	RM, ANOVA
22	Panicle length	cm	1995	III, V	-	RM
23	Panicle weight	g panicle <sup>-1</sup>	1995	III, V	-	RM

† RM repeated measurement

A new approach was also used to evaluate the results discussed in papers I-V. Variation for elite and wild material for each trait was established with common parameters: mean, standard deviation, median and quartile-range. Parameters were calculated separately for each year because of the excessive growth of RCG in the initial production years. Variation of the normally distributed trait was examined using mean and standard deviation and variation of skewed data with median and quartile-range. Standard deviation and quartile range were established both with the effects from block and population and without those effects.

## 4. RESULTS

Variation in many agronomic traits was studied: plant development, plant height and stem elongation (I), biomass related variables (II) and seed production variables (III, IV). Finally, agronomic traits were incorporated to identify the best populations for non-food, forage and seed production, and suitable breeding methods were suggested (V).

### 4.1. Growth related traits

Six developmental stages were determined for RCG and the requirement for effective temperature sum was calculated at each stage from the beginning of the growing season. The first stage determined was flag leaf emergence, which was reached approximately 46 days from the beginning of the growing season, when the approximate temperature sum was 266 °C dd. Anthesis began 65 days from the beginning of the growing season and took 16 days. Seed ripened approximately 95 days from the beginning of the growing season, when the temperature sum was 737 °C dd, and took 14 days to reach maturity. The differences in development among the groups of RCG were statistically significant. Cultivars and breeding lines reached each stage earlier than local groups. Compared with cultivars inflorescence emergence in local populations occurred approximately six days later, seven days later complete anthesis was reached and four days later seed ripened. Groups VIII and X, from northern Finland, were the latest to mature. However, the northernmost group X developed fastest until inflorescence emergence, together with cultivars and breeding lines. Flowering of cultivars and breeding lines took approximately two days less compared with that in wild groups. However, maturing of cultivars took three days more than maturing of wild groups. Groups VI and VII from East Finland had the shortest maturing time among the local groups.

The average plant height of RCG was 76 cm at the time of flag leaf emergence, 135 cm at the beginning of anthesis and 170 cm at seed ripening. At that time, 98% of the maximum plant height was reached. According to logistic growth curves, the theoretical maximum plant height was 181 cm in 1995 and 166 cm in 1996. The increment in plant height was also determined between the six stages of development. The average stem elongation was less than half (76 cm) at the time of flag leaf emergence and 94 cm from flag leaf emergence until seed ripening. Growth curves estimated greatest stem elongation around inflorescence emergence, after which the elongation slowed. The difference in plant height was significant among the groups at each developmental stage. Cultivars and breeding lines were approximately 16 cm taller than wild populations at time of seed ripening. At that time, groups VI and VIII were tallest among the wild groups and group X shortest. However, local groups, especially VIII and IX from northern Finland, were taller than

cultivars before anthesis. Cultivars and breeding lines still exhibited the greatest rate of stem elongation from flag leaf development until anthesis and the northernmost group (X) the lowest. Although group VIII was tall, it had low elongation rate. Likewise, group IX was among the tallest during early development, but maximum plant height was relatively low.

The overall biomass yield of RCG was  $10 \text{ t DM ha}^{-1}$  between 1996 and 1998. The difference in biomass yield among the groups was statistically significant each year. Cultivars and breeding lines had the highest yield, although breeding lines slightly exceeded cultivars at the fourth harvest. The southernmost group (III) produced the highest biomass yield among the wild groups. All groups differed significantly from the northernmost group, X, which produced the lowest yield. The average proportion of plant fractions in a stem was 59% straw, 23% leaves and leaf sheaths, 7% nodes and 5% shoots. The difference in plant fractions among the groups was significant. Straw fraction was highest among the wild groups (IV, VIII and IX) and lowest for breeding lines and the northernmost group (X). Fraction of leaves and leaf sheaths was highest for the northernmost group (X) and lowest among cultivars and breeding lines. Node fraction ranged from 6 to 8% depending on group. Shoot fraction was highest for cultivars and breeding lines (16%) and only 0-8% for the wild groups. Cultivars and breeding lines had also higher shoot number per stem than local groups.

Node number of RCG was counted several times during the growing season and each time significant differences were established among the groups. RCG had approximately 5 to 6 nodes per stem at the beginning of anthesis. In 1995 plant development was faster than in 1996: node number was already 4.5 at the beginning of June, whereas in 1996, at the same time, node number was only 1.1. LAI was measured eight times in 1995 and four times in 1996. In 1995 the mean value for LAI was 4.7 at its highest during the beginning of anthesis. In 1996 the highest value was 6.9, recorded at the time of inflorescence appearance. Furthermore, in 1995 LAI was estimated for each group as a function of accumulated temperature sums ( $^{\circ}\text{C dd}$ ) by quadratic polynomials. Groups differed significantly from each other in shape of profiles, which consisted of three parameters: constant, linear and quadratic terms. Cultivars, breeding lines and wild group VI had generally low values for LAI. The wild group VIII had the highest value for LAI, but it was reached after a relatively long time. Wild group IX also had a high value for LAI, but it decreased more rapidly after peaking than for group VIII. The northernmost group X had a similar profile to cultivars, breeding lines and group VI, but higher LAI values. The average overwintering ability of RCG was 90% over four years, although it decreased from 97% to 85% between 1995 and 1998. The difference in overwintering ability among groups of RCG was significant. Wild groups overwintered better than elite material. The

northernmost group X had the highest overwintering capacity (95%) compared with cultivars, which had the lowest (85%).

Factor analyses included 12 variables from which three factors were retained. The factors were termed “high biomass yield”, “leaf-shoot relationship” and “fast development” and they accounted for 45% of the variance. The first factor explained 21% and indicated a positive relationship between biomass yield, panicle number, plant height, straw fraction and node fraction. Negative loadings came from LAI, shoot number and shoot fraction. The second factor revealed the negative relationship between leaves and shoots: positive loadings came from shoot number and shoot fraction, whereas negative loadings came from LAI and leaf fraction. The third factor indicated a connection between good overwintering and high node number, whereas a high negative loading came from seed ripening.

Correlation analysis between variables revealed that biomass yield was positively correlated with panicle number, plant height, straw fraction and node fraction. Correspondingly, biomass yield was negatively correlated with LAI and shoot fraction. Furthermore, panicle number was positively correlated with plant height, node number, straw fraction and node fraction. Panicle number was negatively correlated with shoot number, shoot fraction, LAI and leaf fraction. The highest positive correlation was found between shoot number and shoot fraction (0.64). LAI and leaf fraction were also positively correlated as well as LAI and shoot fraction. The highest negative correlations were found between straw fraction and shoot fraction, between shoot fraction and leaf fraction and between biomass yield and LAI. No significant differences were established for correlations between advanced and wild material.

#### **4.2. Seed production traits**

The average seed yield was highest at 11-13 DAA at 0.34 g per panicle. At that time artificial seed shattering was 7%, thousand seed weight 0.76 g, seed germination 83%, panicle length 13.3 cm and empty panicle weight 0.20 g. The average panicle number was 380 m<sup>-2</sup>. All the groups performed best at first harvest, except cultivars, which had their highest seed yield at second harvest (0.47 g per panicle). At first harvest the southernmost group (III) had the highest seed yield, although it was not significantly different from that of other high yielding groups. However, group III had significantly higher thousand seed weight than cultivars. Overall the approximate seed shattering was lowest at first harvest. The northernmost group (X) had the lowest seed shattering, and group IX the highest. At first harvest cultivars and breeding lines differed from each other only in panicle length and in panicle number: breeding lines had longer panicles, but cultivars had higher panicle

number. At first harvest cultivars and breeding lines did not differ from wild germplasm in seed shattering, thousand seed weight and panicle length. Wild groups III, IV (southern Finland) and IX (Kainuu) had significantly higher values than other wild groups for seed yield, thousand seed weight and germination. They also differed from each other in panicle number. Overall, first harvest was best for RCG. Furthermore, the differences between elite and wild germplasm were least marked at first harvest.

During the eight harvests (11 to 46 DAA) the average seed yield was 0.13 g per panicle, seed shattering 11%, thousand seed weight 0.75 g, germination 83 %, panicle weight without seeds 0.18 g and panicle length 12.7 cm. Cultivars and breeding lines differed significantly from the wild germplasm for each trait measured, except panicle length. Furthermore, cultivars and breeding lines differed from each other in seed yield and seed shattering: cultivars had higher seed yield and lower shattering than breeding lines. Wild groups III, IV and IX had significantly higher seed yield, thousand seed weight and germination than other wild groups. Seed yield decreased steadily after each harvest until the sixth harvest, after which it was very low and further decreases were negligible. During eight harvests cultivars shattered significantly less than other groups, except the northernmost group (X). Both differed significantly from the easternmost group (VI), which had the highest seed shattering. Cultivars, breeding lines, and groups III and IV from the southern and western coasts had the highest thousand seed weights and they differed significantly from the group from central Finland (V), which had the lowest thousand seed weight. Seed germination was highest with cultivars and breeding lines and they differed significantly from wild groups. Among wild groups VIII and IX had the highest seed germination. Cultivars had the heaviest panicles and they differed significantly from the wild groups (V, VIII, X) with the lightest panicles. Cultivars and the group V from central Finland had short panicles and they differed significantly from the groups III, VI and VII, which had the longest panicles. The northernmost group (X) differed from all other groups having the shortest panicles. Cultivars, breeding lines and groups III and VI had the greatest panicle number. Wild groups III and VI differed significantly from other wild groups. The final harvest was performed 72 to 90 DAA. At that time cultivars had considerably higher seed yield than any other group. Most of the seeds had shattered by then so that shattering was only 7%. Other traits were at their lowest values at the final harvest.

Most of the traits studied were correlated. High seed yield was associated with a high number of long and heavy panicles, especially at first harvest. Panicle length and weight were positively correlated. A significant correlation was also found between seed yield, seed germination and thousand seed weight. Thousand seed weight and germination were weakly correlated except at first harvest. Seed yield was negatively correlated with seed

shattering, excluding the last harvest. The greater the weight and/or length of the panicle, the greater was the number of panicles  $\text{m}^{-2}$  and thousand seed weight. As a whole, seed production traits were not correlated with coordinates of the collecting site. Only slight correlation was established between longitude and seed yield as well as between longitude and seed germination. Populations from western Finland had higher seed yield and better germination than other wild populations. Seed production traits were not correlated with effective temperature sum ( $^{\circ}\text{C dd}$ ) over the long-term (1951-1980). The only significant correlation was established between germination and temperature sum.

Seed production traits of two cultivars, 'Palaton' and 'Venture', were studied more intensively during 1995-1998. Effects of harvest time on seed yield, thousand seed weight and seed germination were evaluated for 'Palaton'. Year had a sizeable effect on seed yield, which was only  $100 \text{ kg ha}^{-1}$  on average. Seed yield was at its highest (200 to  $300 \text{ kg ha}^{-1}$ ) in the second year after establishment. The favourable harvest time for 'Palaton' was determined to be 15 days after anthesis. The effect of storage time (19 months) and storage temperature ( $+20^{\circ}\text{C}$  and  $+6^{\circ}\text{C}$ ) was investigated for 'Venture'. Germination capacity remained relatively high (85%) during the whole testing period. Storage temperature had no significant effect on final germination, but warmer storage speeded up early germination rate as recorded after 7 days of germination.

#### **4.3. Best populations for non-food, forage and seed production**

When high biomass yield or seed yield was used as the only selection criterion, cultivars and breeding lines were best. However, agronomic performance is a sum of several traits and therefore an index was calculated for each end-use. The most promising population according to the established non-food index was the Finnish breeding line, Jo 0510, which had a very high biomass yield, was tall, early maturing and had many panicles. In general, cultivars and breeding lines had above average non-food indices mainly because of their high biomass yield. Population RH26B from Helsinki was the best of the local populations according to the non-food index. It had high biomass yield, a moderate amount of straw and many panicles. Also local population RH50 had a high straw proportion associated with quite high biomass yield.

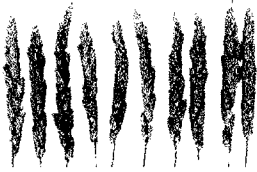
Local Finnish populations were among the best according to forage index. Highest forage index was calculated for RH13, which had moderate biomass yield, high LAI value, high shoot proportion and high panicle number. Local populations, RH22, RH27, RH46, RH83, RH87, and the Finnish breeding line Jo0150, had also high forage index. From the elite material, cultivars 'Motterwitzer' and 'Pervenets' and breeding line SW5 were among the twenty best.

Best seed production indices were found from local populations RH26B from Helsinki and RH9 from Pyhäranta. These populations had both high seed yield and high yield quality. Other local populations among the best ten were RH47, RH50, RH33 and RH85. From the selected material the cultivars 'Palaton' and 'Vantage', as well as breeding lines SW91065 and Jo0510, were the best. Seed production indices of elite material were generally higher than those of local populations. Panicles from the best populations in seed production index were visibly larger and longer than panicles associated with the lowest seed production index (Figure 2).

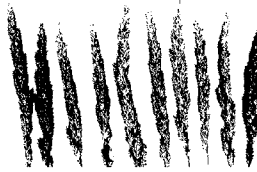
Some populations had both high seed production and high non-food indices. Those were local populations RH26B, RH13, RH36, RH50 and RH87, breeding lines Jo0510, SW91065, SW91066 and cultivars 'Palaton', 'Pervenets', 'Vantage' and 'Venture'. Populations with high seed production index and high forage index were local populations RH13, RH27, RH47 and RH87, breeding line Jo0510 and cultivars 'Motterwitzer' and 'Pervenets'. Populations RH13, RH87, Jo0510 and cultivar 'Pervenets' had high indices for each end-use.

## Best populations

RH26B



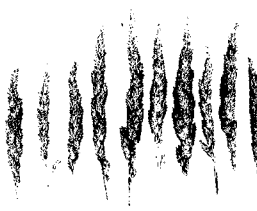
RH9



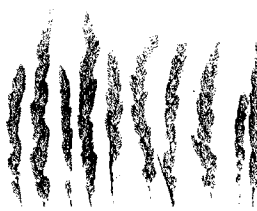
SW91065



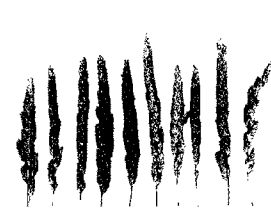
RH85



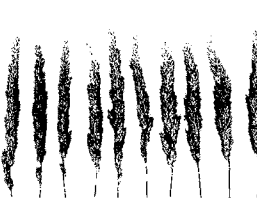
RH33



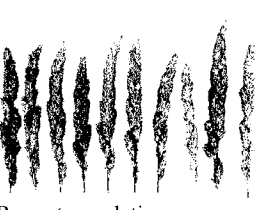
Vantage



RH50



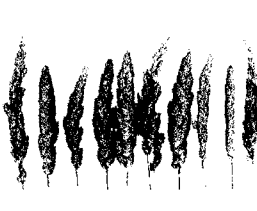
RH47



Jo0510

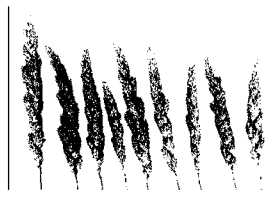


Palaton

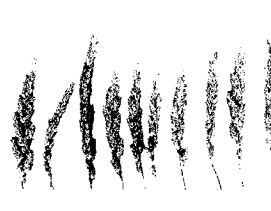


## Poorest populations

RH4



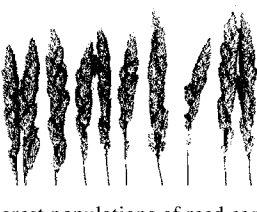
SW1



RH40



RH20



RH89

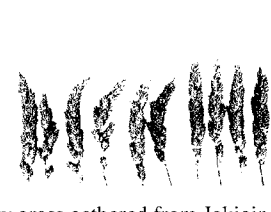


Figure 2. Panicles of best and poorest populations of reed canary grass gathered from Jokioinen, Finland in August 1995 according to seed production index

#### **4.4. Supporting data**

Mean, median, standard deviation and quartile-range for each trait were calculated separately for elite and wild germplasm in order to describe the variation (Table 3). Both the elite and wild populations were variable for each trait. The amount of variation was dependent on trait. The most marked difference between elite and unimproved material was found for traits related to seed production. The variation in seed shattering was excessive for cultivars and breeding lines, whereas wild populations exhibited higher variation for seed yield, thousand seed weight, germination, panicle weight and panicle number. In biomass yield cultivars and breeding lines had higher variation than wild populations in the first production year (1995). Subsequently variation was quite similar, although elite material had higher biomass yield than wild populations. No difference between elite and wild populations was found in variation of plant proportions, except shoot proportion, where wild populations had manifold variation compared with elite lines. Wild populations had also higher variation in shoot number. Variation in plant development was quite low and there was no difference between elite and wild populations.

Table 3. Parameters describing variation in agronomic traits of cultivars and breeding lines and wild populations of reed canary grass

No	Trait	Year	mean	std†	std‡	median	qrange†	qrange‡		
<b>1</b>	<b>Biomass yield (kg DM ha<sup>-1</sup>)</b>	Cultivars and breeding lines	1995	1 967	1 391	763	1 675	1 376	506	
			1996	9 558	2 356	1543	9 710	2 292	1571	
				1997	12 475	2 749	1665	12 409	2 607	1414
				1998	16 423	3 397	2463	16 677	2 985	2489
		Wild populations	1995	1 418	903	527	1 271	751	424	
	1996		8 254	2 330	1558	8 094	2 009	1397		
	1997		10 550	2 940	1568	10 252	2 983	1512		
	1998		13 579	3 359	2440	13 782	3 264	2211		
	<b>2</b>	<b>Proportion of straw (%)</b>	Cultivars and breeding lines	1996	52.8	4.6	3.6	53.0	4.3	3.4
			Wild populations	1996	58.6	6.1	4.5	59.1	5.3	3.2
<b>3</b>	<b>Proportion of leaves and leaf sheaths (%)</b>	Cultivars and breeding lines	1996	17.8	3.9	2.2	17.2	4.4	2.1	
		Wild populations	1996	24.0	5.2	2.8	23.2	5.3	2.7	
<b>4</b>	<b>Proportion of nodes (%)</b>	Cultivars and breeding lines	1996	6.3	1.2	0.7	6.3	1.1	0.7	
		Wild populations	1996	7.2	1.5	0.9	7.1	1.5	1.0	
<b>5</b>	<b>Proportion of shoots (%)</b>	Cultivars and breeding lines	1996	16.5	6.5	3.7	16.8	5.7	3.7	
		Wild populations	1996	5.1	5.5	2.4	2.9	5.0	1.8	
<b>6</b>	<b>Shoot number (per stem)</b>	Cultivars and breeding lines	1996	2.1	0.57	0.30	2.1	0.40	0.31	
		Wild populations	1996	0.89	0.65	0.32	0.76	0.76	0.29	
<b>7</b>	<b>Node number (per stem)</b>	Cultivars and breeding lines	1995§	6.1	0.39	0.26	6	0.30	0.22	
			1996¶	5.5	0.43	0.32	5.4	0.37	0.33	
		Wild populations	1995§	6.1	0.44	0.26	6	0.44	0.29	
			1996¶	5.7	0.51	0.32	5.6	0.44	0.33	
<b>8</b>	<b>Panicle number (m<sup>-2</sup>)</b>	Cultivars and breeding lines	1995	481	126	79	482	120	78	
			1996	485	167	130	467	174	129	
			1997	446	139	87	422	134	86	
			1997	319	149	77	304	174	72	
		Wild populations	1995	455	158	72	464	138	61	
			1996	341	189	101	319	190	78	
			1997	319	149	77	304	174	72	
<b>9</b>	<b>Leaf area index§</b>	Cultivars and breeding lines	1995	4.14	0.71	0.39	4.06	0.74	0.36	
			1996	5.74	0.74	0.47	5.78	0.83	0.49	
		Wild populations	1995	4.87	0.87	0.39	4.86	0.99	0.41	
			1996	6.44	0.95	0.53	6.50	0.90	0.57	
<b>10</b>	<b>Emergence of flag leaf (dd)</b>	Cultivars and breeding lines	1995	244	18.9	9.9	237	13.9	8.6	
			1996	245	15.4	8.1	244	10.1	7.1	
		Wild populations	1995	273	20.8	11.5	278	22.1	10.0	
			1996	271	16.9	8.0	277	12.5	9.1	
<b>11</b>	<b>Inflorescence visible (dd)</b>	Cultivars and breeding lines	1995	269	22.3	12.9	258	30.1	12.5	
			1996	293	18.7	10.7	288	20.1	11.6	
		Wild populations	1995	298	23.9	13.1	298	17.0	12.8	
			1996	325	24.8	10.3	323	22.8	10.4	

<b>12 Emergence of infloresc. (dd)</b>							
Cultivars and breeding lines	1995	335	31.8	15.0	327	28.4	14.2
	1996	341	22.1	10.8	341	17.1	8.7
Wild populations	1995	384	26.9	13.9	384	21.4	12.4
	1996	380	22.3	10.4	381	17.7	10.7
<b>13 Beginning of anthesis (dd)</b>							
Cultivars and breeding lines	1995	392	21.8	10.8	384	21.4	8.7
	1996	421	15.5	8.3	416	12.6	6.6
Wild populations	1995	434	30.9	17.7	461	41.7	11.3
	1996	452	17.6	9.5	452	20.7	8.8
<b>14 Completed anthesis (dd)</b>							
Cultivars and breeding lines	1995	517	35.7	15.4	517	25.3	8.2
	1996	584	37.6	17.3	578	31.8	18.5
Wild populations	1995	568	33.3	14.3	578	41.6	12.0
	1996	630	23.0	12.0	636	24.1	11.0
<b>15 Seed ripening (dd)</b>							
Cultivars and breeding lines	1995	664	32.8	10.2	642	21.5	6.9
	1996	737	24.6	12.6	729	27.8	11.4
Wild populations	1995	709	28.8	12.9	721	40.0	8.5
	1996	784	25.3	10.3	780	19.4	11.4
<b>16 Plant height (cm)</b>							
Cultivars and breeding lines	1995	184	9.9	5.9	186	10.1	5.8
	1996	180	13.1	5.9	182	9.4	5.3
Wild populations	1995	176	11.2	5.2	176	10.5	5.3
	1996	162	16.5	5.8	163	16.6	5.4
<b>17 Overwintering (%)</b>							
Cultivars and breeding lines	1995	92	5.4	4.0	94	3.0	3.3
	1996	81	11.8	9.4	83	11.1	8.2
	1997	85	6.8	5.4	85	7.4	5.7
	1998	83	8.3	6.9	85	7.4	5.5
Wild populations	1995	97	3.3	2.1	97	2.2	1.7
	1996	90	9.1	6.0	92	7.4	5.3
	1997	85	7.4	5.8	85	7.4	5.2
	1998	84	8.1	6.2	85	7.4	5.7
<b>18 Seed yield (g panicle<sup>-1</sup>)</b>							
Cultivars and breeding lines	1995	4.2	0.88		4.1	0.80	
Wild populations	1995	3.4	1.22		3.0	1.15	
<b>19 Seed shattering (%)</b>							
Cultivars and breeding lines	1995	6.9	5.36		5.6	6.93	
Wild populations	1995	8.1	5.41		7.9	4.95	
<b>20 Thousand seed weight (g)</b>							
Cultivars and breeding lines	1995	0.76	0.12		0.73	0.09	
Wild populations	1995	0.76	0.18		0.71	0.13	
<b>21 Seed germination (%)</b>							
Cultivars and breeding lines	1995	86	7.3		86	7.0	
Wild populations	1995	81	13.7		85	17.8	
<b>22 Panicle length (cm)</b>							
Cultivars and breeding lines	1995	13.5	1.23		13.6	1.21	
Wild populations	1995	13.4	1.64		13.5	1.47	
<b>23 Panicle weight (g panicle<sup>-1</sup>)</b>							
Cultivars and breeding lines	1995	0.23	0.035		0.23	0.021	
Wild populations	1995	0.20	0.042		0.19	0.049	

† standard deviation and quartile range with the effects from block and populations

‡ standard deviation and quartile range without the effects from block and population

§ beginning of anthesis

¶ complete anthesis

## 5. DISCUSSION

Results from this study revealed variation in RCG germplasm for each of the 23 traits studied (I, II, III). Hence, RCG represents a promising species for breeding for different end-uses: non-food, forage and seed production. In most cases, the amount of variation of cultivars and breeding lines was similar to that in wild populations. Wild populations showed higher variation in seed production traits and shoot number, whereas elite populations were more variable in seed shattering. A high amount of genetic diversity was also revealed both for RCG cultivars and local populations in another study, where isozymes were used in conjunction with morphological traits (Gifford et al. 2002). Thus, a hundred years of RCG breeding has not greatly reduced variation among cultivated populations and they are probably still genetically close to wild types. Furthermore, wild populations showed geographic distribution on the basis of agronomic traits; they were more similar within an area than among areas. Information on geographic variation will be helpful in locating particular traits of interest for breeding and targeting new germplasm collections. RCG has primarily been growing wild in Finland and its cultivation has been minimal to date. Some of the wild populations could be escapees from cultivars or crossings of cultivars and wild populations. Wild populations from the coastal areas probably originated from overseas, whereas other populations originated from the east (Russia). After the morphological and phenological study, which was the major focus of this work, it would be possible to identify molecular markers unique to different RCG populations and reveal the genetic distances among populations. By using  $F_{ST}$  (molecular) and  $Q_{ST}$  (quantitative traits) statistics it should be possible to study the influence of selection on important agronomic traits (Merilä and Crnokrak 2001, McKay and Latta 2002).

Substantial genetic diversity of RCG has also been revealed in previous studies. Thus, insufficient variation will not be an obstacle in RCG breeding. The high variability of RCG is partly due to its natural cross-pollination habit, which makes it highly heterozygous. Furthermore, cultivars of RCG are composed of several parents or populations, which increase the amount of variation. Early studies found variation in many agronomic traits for populations selected in USA (Baltensperger and Kalton 1958, 1959). Growth traits of natural Canadian populations were variable both within and among populations (Sachs and Coulman 1983). Similarly, genetic variability was found for mineral content in the USA (Hovin et al. 1978), for morphological and chemical characters in Sweden (Lindvall 1997) and for seed germination, dormancy and agronomic performance in Norway (Landgraff and Junttila 1979, Berg 1980, 1982). Variation was also found in alkaloid type and concentration, forage yield and quality and seed yield components (Østrem 1987, Østrem 1988ab). In an combining ability experiment of parents and progenies, cell wall

constituents and associated quality traits were found to vary (Marum et al. 1979a). However, in most cases it was due to non-additive genetic variance, which may be difficult to capture in a cross-pollinating species. The variation examined in this study among groups was phenotypic, which resulted from genotypic and environmental effects, from error inherent in the techniques used, and from intra-population variation within a group. However, populations grew under quite similar conditions in the field and thus, the variation was assumed to be largely genetic.

The difference between elite and wild germplasm in agronomically important traits was well demonstrated. As expected, cultivars and breeding lines differed from wild material by having higher biomass yield and higher panicle number, and being taller and earlier in their development. Elite material also had better seed production properties than wild populations: higher seed yield and seed germination and lower seed shattering. In general, elite germplasm performed very well under Finnish long-day conditions. For example cultivars 'Barphal' (Netherlands), 'Motterwitzer' (Germany), 'Pervenets' (Russia) and 'Vantage' (USA) were the highest yielding, whereas other North-American cultivars performed moderately. Current forage cultivars have been expected to be unsuitable for non-food production, for example, by being too leafy or having too high a mineral content. However, when RCG was harvested in spring the leaf mass was naturally low following shattering during winter and harvest. According to the results of this study, leafiness was not a problem with spring harvested forage cultivars, and leaf proportion of wild populations was even higher. Furthermore, variation in mineral content (K, Si, Cl) was found to be significant among populations in Finland (Sahramaa and Hömmö 2000). Low mineral content is one breeding aim, but differences are likely to be so minor, that breeding for reduced mineral content will probably not be feasible. Although currently used forage cultivars are not the most appropriate for non-food production, this study indicated their good performance and showed that they represent potential breeding material for Finnish conditions.

Wild populations were inferior to elite material as a whole, but some of them possessed a desirable combination of traits for different end-uses and might therefore be useful in breeding. For instance, wild populations had higher overwintering ability, being evidently due to natural selection in local Finnish populations. They represent winter-hardy parental material for high latitudes. In experiments conducted in Alaska, RCG cultivars from Canada and USA had very poor winter-survival compared with material originating from northern Norway and Sweden (Klebesadel and Dofing 1991). At the seed ripening phase some local populations were taller than elite material. This indicates potential for increased plant height, and thus, for increased biomass yield, as those traits were shown to be associated with each other (II). Surprisingly, biomass yield of wild RCG was quite high

without any breeding efforts. Groups of wild populations from southern and western coasts of Finland had the highest biomass yields and groups from northern Finland the lowest, which was also the case in a study of Swedish local populations (Lindvall 1997). Some wild populations had higher straw proportion associated with high biomass yield, which is desired in non-food production. Overall, wild populations had higher straw and leaf proportion in their phytomass, higher node number and greater leaf area. Wild populations had also markedly fewer branches originating from stem nodes than cultivars measured from spring harvested material. Stem branches develop when RCG is not cut during late summer (Evans and Ely 1941). Thus, wild populations are potentially useful in non-food breeding programmes, where unbranched stems are sought.

Forage cultivars and breeding lines were well adapted to grassland management and they appeared to be a fairly homogeneous group as compared with wild populations. Finnish wild populations have evolved through natural selection during decades and they were well adapted to local conditions and to low-input environments. Cultivars originated from many different countries and therefore their homogeneity as a group may indicate wide adaptation of this species. Another indication of wide adaptation is that RCG grows in diverse environments, from dry to wet conditions. Perennial forage crops are usually bred for wide adaptation; for different habitats, for different harvest times and for different farming systems. Species taken from south to north differ from those adapted to northern growing conditions in many ways. They often have enhanced vegetative growth, retained or later flowering and do not show acclimation by preparing to cease growing in the autumn (Tigerstedt 1994). Similarly, species taken from north to south mature faster as they are adapted to a shorter growing period. In this study, the situation was partly *vice versa*: foreign cultivars and breeding lines from lower latitudes had the highest biomass yield, though they were also the earliest to mature. Wild populations from Lapland had higher rates of stem elongation during early growth stages (pre-anthesis), but they were not the tallest ones. They were also the latest maturing. Obviously, they had lower stem elongation potential and no ability to benefit from the longer growing season in southern Finland. This effect may arise from complicated G x E interaction, where photoperiodic and thermoperiodic adaptation interact with a novel environment.

Ideal plant types, ideotypes, have been described for many crops and now (V) also for RCG. An ideotype for non-food production should have a tall, strong, unbranched stem and few small leaves. A plant stand should contain many stems, be winter hardy, have low mineral content and be resistant to pests and diseases. In seed production the main breeding objectives are high seed yield, even seed ripening, low seed shattering, good germination and high thousand seed weight (Sahramaa et al. 1997). The emphasis of this study was to characterise populations for non-food and seed production, but some ideotype

characteristics were also provided for forage breeding. The most suitable populations for each end-use were described using an index composed of different traits (V). High non-food or forage indices were associated with good seed production in some populations.

Breeding efforts should be directed towards higher biomass yield contributing to improved economics of non-food production at the farm level (Klemola et al. 2000). However, biomass yield is a complex trait, quantitatively inherited, with low heritability. In this study, many traits were connected with high biomass or seed yield. In other studies their heritabilities were shown to be quite high. For example, Sachs and Coulman (1983) calculated broad sense heritabilities for local accessions. They were 0.54 for plant height, 0.49 for panicle number, 0.94 for heading date, 0.47 for spring vigour and 0.67 for seed retention. In a Norwegian study, broad sense heritabilities for alkaloid concentration ranged from 0.19 to 0.70 for populations from different origins (Østrem 1987). Broad sense heritabilities over clones were estimated at 0.50 for grass yield, 0.77 for earliness, 0.49 for panicle number and 0.41 for seed yield (Østrem 1988ab). Thus, ideotype breeding, modifying individual specified traits, is positively associated with yielding ability (Rasmusson 1987) and should lead to cultivars with higher biomass yield.

In general, breeding methods for RCG are similar to those for other herbage grasses. Open-pollination of individual plants, polycross and pair-matings are the most common breeding systems for perennial forage grasses (Aastveit and Aastveit 1990). In addition to synthetics, promising local populations identified in this study serve as well-described candidates for developing cultivars *per se* or for single crosses. Comparative trials in different locations are needed to establish their value in cultivation before release. This experiment was conducted in one location in southern Finland and therefore, no G x E could be studied. However, associations among collecting site variables (latitude, longitude, heat units) of wild populations and seed production traits was tested without finding significant associations. Many studies of G x E have been documented for RCG. In American studies, G x E interaction was found for forage yield (Barker et al. 1981), for digestible dry matter, crude protein, plant height and resistance to tawny blotch (*Stagonospora foliicola* Bres.) (Zeiders and Sherwood 1985) and for seed production traits (Rincker et al. 1977). In Finland fifteen RCG breeding lines were studied at three experimental sites between 60-64°N (Sankari and Mela 1998). Although targeting of best populations to different locations was not possible, inferior lines were shown to be the same at all sites. In another experiment in the USA diverse environments had no great effect on alkaloid type or concentration (Marten et al. 1973), whereas in Norway a humid environment resulted in lower alkaloid concentration than a dry environment (Østrem and Marum 1989). Studies on G x E suggest that some populations tend to be superior or

inferior in certain traits regardless of the environment. This suggests that RCG breeding should target only a few cultivars to be used over relatively large areas.

## 6. CONCLUSIONS

RCG is a potential crop for energy, paper and forage production under northern conditions. This study included 53 wild populations of Finnish origin from 60-66°N, 8 cultivars from central Europe and North America (from regions 10-15° south of Finland) and 14 selected breeding lines of various origins. The RCG material comprising this study represented realistic coverage of RCG genetic resources. This study may thus form the basis for a full-fledged breeding programme for improved non-food and forage cultivars. The present study of 23 agronomic traits revealed that both elite and wild populations exhibited variation for each trait. Cultivars and breeding lines had good biomass and seed yield traits. Wild populations offered a versatile source for breeding for improved winter-hardiness, longer straw, greater leaf proportion and more unbranched stems. Some wild populations were also high biomass producers. Geographic variation for wild populations should be exploited in locating particular traits of interest for breeding and targeting new germplasm collections. At this point it is possible to select populations for cultivar trials or pick components to form a synthetic cultivar. A first selection should be undertaken, bearing in mind that the species is allogamous and all entries are basically populations and grown in one location. Thus, selection should be mild, discarding perhaps 50% of the poorest performers. Replicated trials of the best populations should be carried within a tentative cultivation region. The new trials form the basis for the estimation of G x E interaction and ultimately stability. In addition to simple breeding, this material would be useful for analysis of genetic diversity at the molecular level. This then would form the basis for  $F_{ST}$ - $Q_{ST}$  analysis that could provide information not only on genetic diversity but also to indicate how selection on quantitative traits has affected the populations.

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