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Morphological descriptors of Christmas rose (*Helleborus niger* L.)

Andrej ŠUŠEK*

[1]University of Maribor, Faculty of Agriculture, Vrbanska 30, 2000 Maribor, Slovenia

The Christmas rose (*Helleborus niger* L.) is becoming an important ornamental plant. In the future, a systematic genetic improvement will probably become inevitable and therefore the characterisation of the naturally grown populations and the accessions within germplasm collections will be extremely important for the breeders. We followed the idea that characterisation of genetic materials has to involve highly heritable morphological traits which are important for the selection and are easy for the determination. The study is based on 5-year long observations of the Slovenian and Croatian naturally grown populations and germplasm collections. The paper describes 72 morphological traits that appear to be at least partly genetically controlled and can be used as descriptors of Christmas rose genotypes.

Key words: Christmas rose, *Helleborus niger*, ornamental plant, morphological descriptors

INTRODUCTION

Recent studies indicate that the Christmas roses (*Helleborus niger* L.) are becoming more and more popular on the market as an ornamental plant (van der Meer 1999, Armstrong 2002; Roggendorf 2003). They can be grown as cut-flowers or pot plants and may be forced to flower under greenhouse conditions around Christmas or New Year.

The number of genetically improved varieties of the Christmas rose on the market is limited (Ahlburg 1989; Rice and Strangman 1999). Mclewin (1996) established the following: (i) that there are no well established, distinct cultivars or strains; (ii) that its cultural requirements are not clearly defined, and its horticultural behavior is unreliable; (iii) that the breeding propensities have not been established, and (iv) that there is much more variation in size, form and colour in wild plants than among those in cultivation.

In future, the genetic improvement will probably prove essential, and reliable data about existing diversity and variation within existing germplasm collections will be extremely important for breeders. Christmas rose germplasm collections are rare and often include a limited number of genotypes. Slovenia may play an important role as one potential centre of diversity of the Christmas rose. Its genetic diversity is considered to be relatively high, which was demonstrated by morphological analyses (Šušek et al. 2005). To get more information about the existing variation in Slovenia and other countries, it will be necessary to establish systematic national and international germplasm collections, and to describe accessions according to an appropriate descriptor list.

The variation that is observed among individuals can be the result of their genetic structure, the influence of the environment and the interaction between these factors. Thus, morphological variation will to some degree present underlying genetic variability, but it will also be intertwined with variation caused by various environmental factors. Nevertheless, in the absence of direct genetic methods, morphological variation of a suite of traits is often used to resolve the population structure of a species (Hughes 2001).

Traits that are highly influenced by environment are not reliable for the characterisation of individuals, groups of individuals or populations. Characterisations have to be based on highly heritable traits, called morphological descriptors (Ivančič and Lebot 2000). Morphological descriptors should be scored easily and must have a constant or very similar phenotypic expression in all environments (i.e., high heritability and low environmental influence). For this reason, descriptors for germplasm characterization are those which are not biased by the environment. Meanwhile, descriptors with low (or non-significant) genotype by environment interaction, although they may be affected by the environment, are more important for agronomic evaluation or selection than for classification. Each coded morphological descriptor (e.g. leaf shape) can be translated into a binary data system for the presence or absence of a peculiar trait (0s and 1s). The resulting data matrix can subsequently be computed with statistical analysis, and groups can be identified. Such groups of related morphotypes can be useful for the evaluation of any germplasm collection materials in order to plan genetic recombination (Ivančič and Lebot 2000). In most cases, classifications are based on a few easily recognizable morphological characteristics that allow a rapid overview of variation within the collection.

Correspondence to:
Phone: +386 2 250 58 05
Fax: +386 2 229 60 71
E-mail: andrej.susek@uni-mb.si

Table 1. The taxonomic and morphological traits that can be used as descriptors of *Helleborus niger* genotypes

Trait	Variation range
General characteristic	
Growth vigor:	poor; intermediate; high; very high; other
Growth habit:	erect; semi-erect; semi-prostrate; prostrate (spreading); other
Roots	
Rhizome formation:	poorly developed; intermediate; well-developed;
Maximum length of rhizome (cm):	< 5; 5-10; 11-15; 16-20 ; > 20
Orientation of rhizomes :	horizonatal ; semi-vertical ; vertical
Number of adventitious roots (roots attached to rhizome) :	< 10 ; 11-20 ; 21-30 ; > 30
Leaves	
Number of leaves/plant :	< 5 ; 5-10 ; 11-15 ; 16- 20 ; > 20
Predominant lamina orientation :	vertical; semi-vertical; semi-horizontal; horizontal; horizontal with drooping tip; horizontal with tip pointing upwards; other
Lamina waxiness:	non-glossy (non-shining); glossy (shining); only young leaves glossy; only mature leaves glossy; other
Lamina colour:	yellow; green; pale green; dark green; light purple; dark purple; other
Average number of leaflets:	< 5; 5-10; > 10
Average number of serrated leaflets (leaflets which are not entire):	0; 1; 2; 3; 4; > 5
Shape of terminal leaflet:	elliptic; ovate; obovate; oblong-cuneate; oblanceolate; lanceolate
Terminal leaflet tip:	acute; obtuse; other
Terminal leaflet base:	attenuate; acute; cuneate; oblique;other
Terminal leaflet margin:	entire; crenate; sinuate; dentate; denticulate; serrate; serrulate; other
Distribution of teeth of terminal leaflet:	no teeth; upper 1/3; upper 2/3; on whole margin; other
Number of terminal leaflet teeth:	none; 1-10; 11-20; 21-30; > 30
Maximal width of terminal leaflet (cm)	
Terminal leaflet length (cm)	
Terminal leaflet width : length ratio	
Pigmentation of main leaflet veins (colour chart)	
Terminal leaflet stalk length (cm)	
Petiole	
Petiole junction colour (colour chart)	
Petiole basic colour (colour chart):	colour of basal 1/3rd; colour of middle 1/3rd; colour of top 1/3rd;
Petiole waxiness:	non-glaucous; glaucous; only lower part glaucous; only upper part glaucous; other
Petiole length (cm)	
Fresh petiole sap colour:	transparent; whitish; pale green; light pink, other
Peduncle[1]	
Penducle orientation :	erect; semi-erect; curved
Peduncle length[2]	
Peduncle length/inflorescence length ratio	
Number of bracts :	0-1; 2-3; 4-5; > 5
Shape of bracts:	entire; divided
Margins of bracts:	entire; partly dentate; partly serrate; dentate; serrate; other
Position of bracts:	erect; horizontal; curved; pendulous
Peduncle orientation:	erect; non-erect; curved; other
Peduncle basic colour (colour chart):	colour of top 1/3rd; colour of middle 1/3rd; colour of basal 1/3rd
Inflorescence	
Number of inflorescences per plant:	< 5; 6-10;11-20; 21-30; 31-40; 41-50; > 50
Average number of flowers per inflorescence:	solitary flower; 2; 3; 4; > 4

Trait	Variation range
Flower	
Odour intensity:	absent; very weak; weak; moderate, strong
Odour type:	pleasant; not pleasant; other
Flower shape during anthesis:	flat; flattish; bell-shaped; narrow bell-shaped; other
Position of floral axis:	erect; semi-erect, horizontal; drooping
Flower diameter (cm):	< 4; 4-6; 6-8; 8-10; 10-12; > 12
Flower height (distance from the floral base to the tip) (cm)	
Flower diameter : height ratio	
Number of sepals:	< 5; 5; 6; > 6
Sepal shape:	elliptic; lanceolate; oblanceolate; oblong; obovate; ovate; oval; rhomboid
Sepal margins:	entire; crenate; sinuate; dentate; denticulate; serrate; serrulate;
Colour of sepals (adaxial side) (colour chart)	
Colour of sepals (abaxial side) (colour chart)	
Colour of sepal margins (colour chart)	
Position of sepals:	separated; slightly fused at the base; overlapping; other
Number of stamens:	1-30; 31-60; 61-90; 91-120; > 120
Position of anthers:	forming a circle around female portion; irregularly distributed; other
Filament length (mm):	very short (< 3 mm); short (3-5); intermediate (5-8); long (8-10); very long (> 10 mm)
Anther colour (during anthesis):	light yellow (normal); dark yellow; pink; red; purple; blue or blue-purple; other
Number of carpels:	< 3; 3-6; 7-10; > 10
Colour of carpels at the beginning of anthesis :	light yellow (indicating sterility); pale green (indicating poor vigour); green; purple or purple-red; not uniform; other
Style length:	short (< 1/10 of the pistil height); intermediate (1/10-1/5 of the pistil height); elongated (> 1/5 of the pistil height)
Stigma pigmentation:	light red; red or purple-red; blue or blue-purple; stigmas on the same flower differ in colour; other
Petal shape:	flat; flat with curved margin; tubular; funnel; other
Number of petals:	1-10; 11-15; 16-20; > 20
Petal colour:	yellow; yellow-green; green; green-purple; purple; other
Pollen colour:	light yellow (usual); brownish yellow; red or pink; purple or purple-blue; pollen grains from the same inflorescence differ in colour; other
Fruit	
Fruit formation:	absent; present; rarely present
Mature fruit colour:	yellowish green; green; dark green; green with red patches; red; purple; other
Number of fruits/plant	
Number of seeds/fruit:	< 5; 5-10; 11-20; > 20
Seed	
Seed colour:	light (almost white); light brown; darker brown; black; darker red or purple; colour variations within seeds from the same fruit head; other
Seed shape (fresh seed):	elongated; elliptic; oval; round; irregular; other
Dry seed shape:	elongated; elliptic; oval; conical; irregular; other

[1] mature peduncle (during flowering)
[2] the distance from soil surface to the base of the flower

The descriptors of Christmas rose have not yet been fully defined. The majority of the economically important crops have detailed descriptors lists while for the Christmas rose we have only the list of the key traits. It would be very useful to establish a descriptor list for each of the economically important *Helleborus* species in order to systematise all important and genetically controlled traits. Descriptors are essential for the determination of cultivars and conservation of the germplasm. For this reason it would be very helpful to have reliable data about the variation within cultivated and wild populations.

The main objective of this paper was to prepare the list of morphological traits, which could be used in description of the wild and cultivated genotypes, and as selection criteria in genetic breeding.

MATERIAL IN METHODS

The descriptor list is based on the documented observations of the phenotypic variation within various germplasm collections of wild and cultivated materials of Christmas rose and natural (wild) populations during the period 2000-2005. It included 8 local populations within geographically discrete, wild communities of the Christmas rose: (1) Bizeljsko, (2) Bohinjska Bela, (3) Logarska dolina, (4) Sodražica, (5) Črnomelj, (6) Žumberak, (7) Peca and (8) Rimske toplice. The local Žumberak population is in Croatia near SE border of Slovenia, while the others are in Slovenia.

The assessment of the non-genetic factors was based on the monitoring the variation of a particular trait within individuals (e.g., the number of terminal leaflet teeth was determined on 5 or more leaves on the same plant) and/or within clones. The monitoring the variation within the same individual was more frequently used because the number of clones and the individuals within clones was limited. The traits were determined at the beginning of flowering (Fig. 1).

Colours were determined on plants fully exposed to sun (colour of plants in shade were less intensive).

In order to determine the variation range, the traits were studied on 100 or more individuals and on several locations.

RESULTS AND DISCUSSION

Our study of wild Slovenian and Croatian populations and materials in germplasm collections indicates that there are at least 72 morphological traits that appear to be at least partly genetically controlled and can be used for the characterization of the genetic materials. The proposed descriptor list which consists of 72 taxonomic and morphological traits with their variation ranges is presented in Table 1.

The most reliable traits are: shape of terminal leaflet, terminal leaflet tip, terminal leaflet base, terminal leaflet margin, fresh petiole sap colour, flower shape during anthesis, sepal shape, colour of sepals (adaxial side), colour of sepals (abaxial side), colour of sepal margins, mature fruit colour, seed colour and dry seed shape. Among reliable traits are also growth habit, predominant lamina orientation, lamina waxiness, distribution of teeth of the terminal leaflet, number of terminal leaflet teeth, terminal leaflet width-length ratio, pigmentation of main leaflet veins, petiole junction colour, petiole waxiness, peduncle orientation, number of bracts, margins of bracts, position of bracts, peduncle basic colour, odour type, position of floral axis, position of anthers, anther colour (during anthesis), stigma pigmentation, petal colour and pollen colour. The least reliable traits are growth vigor, maximum length of rhizome, number of adventitious roots (roots attached to rhizome), number of leaves/plant, number of inflorescences per plant,

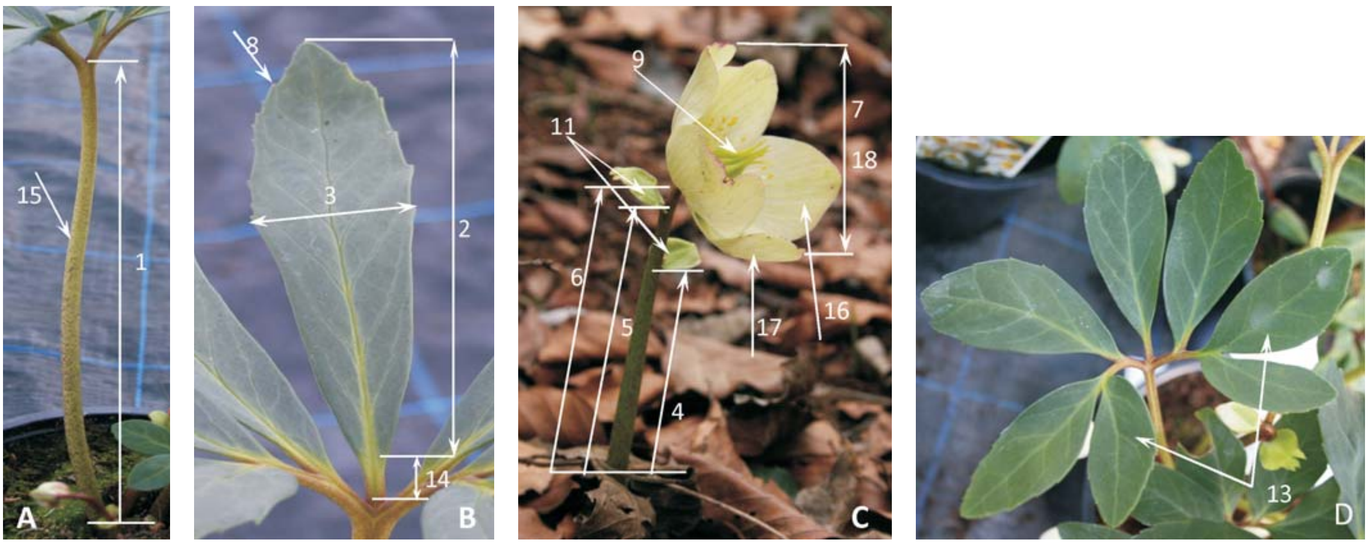

Fig. 1. Diagrams of some traits. (A) 1 - petiole length, 15 - pigmentation of leaf petiole; (B) 2 – length of the terminal leaflet, 3 - maximal width of the terminal leaflet, 8 - number of teeth; 14 - terminal leaflet stalk length; (C) 4 - distance of first bract from the peduncle base, 5 - distance of second bract from the peduncle base, 6 - peduncle length, 7 - floral diameter, 9 - number of carpels, 11 - number of bracts, 16 - colour of sepals from adaxial side (inner part), 17 - colour of sepals from abaxial side (outer side) and 18 - flower shape; (D) 13 – number of serrated leaflets.

flower height (distance from the floral base to the tip). These traits appear to be highly influenced by the environment, especially by soil fertility.

In the future research, it will be necessary to obtain more exact data about the genetic inheritance. The easiest and the fastest way to obtain data about the inheritance of quantitative traits will be to conduct a series of crosses among phenotypically different individuals, create sufficiently large offspring populations and then to calculate the regression of offspring on one parent, or the regression of offspring on mid-parent (Falconer 1981, Ollivier 2002). The easiest way to determine the environmental variance will be to study the variation within clones (i.e. vegetatively propagated individuals which originate from the same plant and are of the same age) grown in different environments. The hybridisation among the members of the same clone will probably result in genetically highly variable offspring populations owing to the predominant heterozygotity of the parental plants.

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