



VARIABILITY OF CHRISTMAS ROSE (*HELLEBORUS NIGER* L.) POPULATIONS AND ITS POTENTIAL USE IN GENETIC BREEDING

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The Christmas rose (*Helleborus niger* L.) is widely distributed in Southern and Central Europe, and Slovenia is probably one of the centers of its diversity. The investigation was aimed at evaluating phenotypic diversity among selected wild populations in Slovenia and neighboring Croatia. Some of the analyzed traits expressed relatively very high variation (e.g., number of leaves per plant, number of inflorescences per plant). The variation ranges of some of the analyzed traits were found to be wider than those mentioned in the literature. Statistical analysis indicated that variability was more expressed within than between populations. In terms of present market requirements, the most valuable populations are Žumberak (Croatia), containing plant material with the longest peduncles and largest flowers, and Peca, containing genotypes with the highest number of inflorescences per plant.

Key words: *Helleborus niger*, Christmas rose, geographical distribution, phenotypic variation, population diversity.

INTRODUCTION

The genus *Helleborus* L. (family Ranunculaceae) comprises about 16 species of herbaceous perennials, which are widely distributed from Southern and Central Europe to Western Asia. The Balkan Peninsula is considered to be the main center of its diversity (Ravnik, 1969; Hegi, 1975). Only one species, *H. thibetanus* Franch., is found outside Europe and Western Asia, on the eastern margins of the Qinghai-Tibet Plateau (Mathew, 1989; Mclewin and Mathew, 1999). The Christmas rose (*Helleborus niger* L.) grows wild in the southern and occasionally the northern Dolomites, Apennines and northwestern Balkans, and in mountain forests of the southern Alps (Ravnik, 1969; Hegi, 1975; Jelitto and Schacht, 1995).

The Christmas rose is known for its early flowering and its ability to bloom in the coldest months of the year when everything else is frozen. It is becoming

more and more popular as a hardy, disease-free, late winter flowering perennial. It can be grown for sale as cut flowers or potted plants, and can be forced to flower under greenhouse conditions around Christmas or New Years (Lamper, 1984, 1985).

The Christmas rose is a rhizomatous, evergreen perennial, 15–30 cm high (Hegi, 1975). The underground part is a short branched rhizome, usually deeply rooted. Young roots are pale brown, while older ones are black/brown. The leaves are evergreen and deeply divided into 5 to 11 segments (Ravnik, 1969), which are usually entire and sometimes serrated toward the tip. The flowers, usually one per stem, are relatively large and flat, and according to Ravnik (1969) their diameter ranges from 6 to 11 cm. The perianth consists of two similar whorls; there are numerous stamens arranged in a spiral, and small green tubular-shaped, shortly stalked nectaries (modified petals) on a cone-shaped receptacle. Five to ten separate carpels form the central

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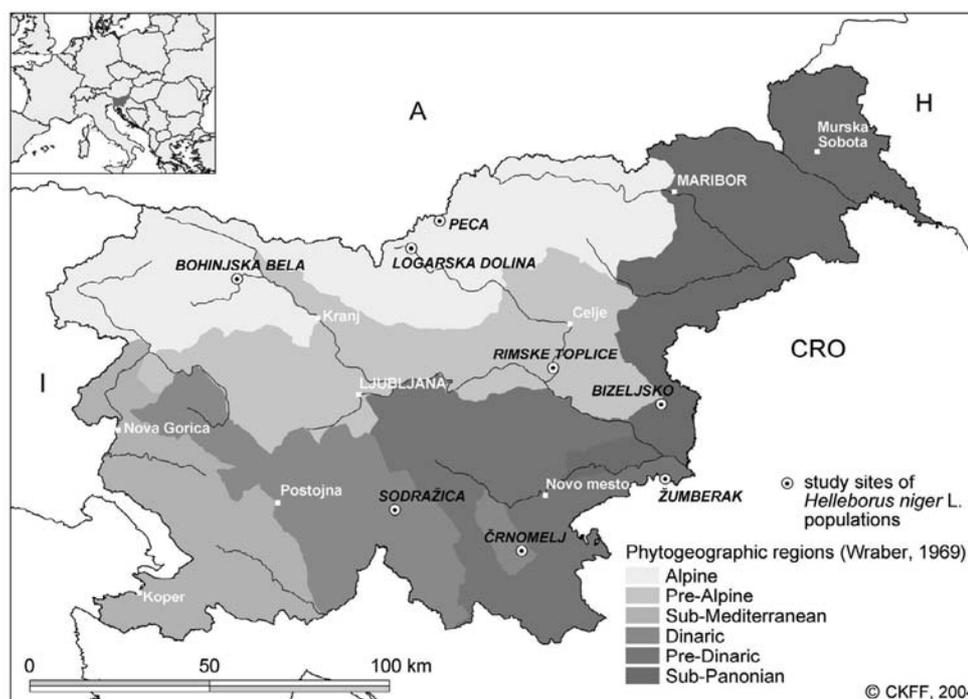


Fig. 1. Phytogeographical division of Slovenia (after Wraber, 1969) and location of study sites. Map adapted by permission of the Slovenian Centre for Cartography of Fauna and Flora (CKFF).

whorl of the floral structure (Trinajstič, 1967; Frankel and Galun, 1977). The flowers usually appear from November to April, depending on the weather. Although usually white with a green "eye," the flowers may be pink on the underside, or may turn pink on both sides as they age.

Slovenia, one of the smallest European countries, seems very rich in terms of biotic diversity, with over 3000 vascular plant species (Martinčič, 1999). The main reasons for such diversity are its geographic location (Fig. 1) and its diverse climate, relief and bedrock (Kladnik, 1996; Ogrin, 1996; Perko, 1998; Andrič and Willis, 2003). The climate is predominantly sub-Mediterranean in the southwest, mountainous in the northwest and moderate continental in the central and eastern parts (Ogrin, 1996). The geology varies from Tertiary flysch and sandstones in the southwest to Palaeozoic and Triassic rocks in the center and north, Jurassic and Cretaceous limestones and dolomites in the south, and Oligocene/Miocene sands and sandstones in the east (Andrič and Willis, 2003). Slovenia has at least six distinctive phytogeographical regions (Wraber, 1969), and *H. niger* is widely grown in all of them. Its habitats, however, appear to be less frequent in the sub-Pannonian and sub-Mediterranean region (Jogan, 2001).

The number of genetically improved varieties on the market is limited (Ahlburg, 1989; Mclewin, 1996;

Rice and Strangman, 1999). The main reason is a lack of genetic resources (sources of genes for specific traits such as specific flower colors, flower size, overlapping sepals, double flowers, plant height, adaptability to the glasshouse environment, and resistance against diseases).

Another reason is the sophisticated and time-consuming breeding process. The main criteria for selection are resistance/tolerance to pests and diseases, vigorous growth, large flowers, long peduncles, ability to flower before Christmas (the achievable price of Christmas rose before Christmas is double the price after the festive season). Production for the market is based largely on seed propagation. To obtain the desired level of phenotypic uniformity, each plant has to be self-pollinated for six or seven generations in order to reach a sufficiently high level of homozygosity (Armstrong, 2002; Roggendorf, 2003). When the plant material is highly homozygous, it can be used for hybridization to form F_1 hybrids. To make a good F_1 hybrid, the breeder has to test the combining ability of the available inbred lines. To make a successful F_1 hybrid usually takes more than ten years.

Genetic improvement is probably inevitable, and reliable data about the existing diversity and variation within existing germplasm collections will be extremely important for breeders. Slovenia may play an important role as one of the centers of diversity.

TABLE 1. Location of study sites and annual precipitation

Location	Phytogeographical region	Coordinates	Altitude (m a.s.l.)	Annual precipitation (mm)
Bizeljsko	Sub-Pannonian	46°01'15" N, 15°38' 15" E	620	1060
Logarska dolina	Alpine	46°25'20" N, 14°40' 45" E	642	1900
Sodražica	Dinaric	45°45'45" N, 14°36' 50" E	775	1488
Bohinjska bela	Alpine	46°21'15" N, 14°05' 15" E	481	1519
Rimske toplice	Pre-Alpine	46°07'15" N, 15°12' 45" E	240	1120
Peca	Alpine	46°29'25" N, 14°47' 40" E	1131	1464
Črnomelj	Pre-Dinaric	45°40'10" N, 15°05' 40" E	640	1250
Žumberak	Pre-Dinaric	45°49'45" N, 15°35' 15" E	363	1142

The main objectives of the presented study are to assess phenotypic diversity between studied natural populations, to identify the most variable traits within each population, and to identify the geographical patterns of variation between the studied populations.

MATERIALS AND METHODS

STUDY SITES

The presented study includes eight locations of naturally grown populations of the Christmas rose: (1) Bizeljsko, (2) Bohinjska Bela, (3) Logarska dolina, (4) Sodražica, (5) Črnomelj, (6) Rimske toplice, (7) Peca and (8) Žumberak. The first seven are in Slovenia, and the last in Croatia along the Slovene border. These locations were selected as a result of a series of preliminary studies involving 73 locations across Slovenia, aimed at collecting basic data about the natural habitats of this species and the possibilities of forming a germplasm collection useful for breeding purposes and eventual marketing. The main criteria for selecting these locations were a large number of plants and high density, except for Žumberak which was chosen because of the extremely tall plants and large flowers there. They are situated in the Alpine (Bohinjska bela, Logarska dolina and Peca), Pre-Alpine (Rimske toplice), Dinaric (Sodražica, Črnomelj), Pre-Dinaric (Žumberak) and Sub-Pannonian (Bizeljsko) phytogeographical regions (Fig. 1, Tab. 1), characterized by predominantly continental to montane climate. The studied populations occupied 0.2–6 ha: Bohinjska Bela 6 ha, Logarska dolina 0.3 ha, Rimske toplice 0.2 ha, Sodražica 0.3 ha, Bizeljsko 1 ha, Peca 3.2 ha, Črnomelj 3.5 ha, and Žumberak 0.3 ha.

STUDIED TRAITS AND DATA ANALYSIS

The assessment of variability included ten morphological traits (six quantitative and four qualitative): pigmentation of leaf petiole, color of sepals (adaxial and abaxial side), flower shape, petiole length (cm), number of leaves, peduncle length (cm), number of carpels,

average pressed/flattened flower diameter (cm), and number of inflorescences per plant.

The total number of plants analyzed was 1670, 210 plants of each population. The plants were selected randomly except that very young, very old, and non-flowering individuals were excluded. The plants were collected during February and March 2002.

Statistical analyses employed Statistica (5.5.99 release). The basic analysis of quantitative and transformed qualitative traits included calculation of means, extremes (maximum and minimum), standard deviations, coefficients of variation (CV) and correlation coefficients. The qualitative traits were transformed to an ordered nominal scale. For example, the color of the abaxial side of sepals was ranked from 1 to 15 (1 = white, 15 = dark brown-purple). Relationships between qualitative traits were checked with the chi-square test. For the quantitative traits, ANOVA was used to verify the significance of differences between populations. Principal component analysis (PCA) was also performed for the same data.

RESULTS

PHENOTYPIC VARIATION IN GENERAL

The highest variation (Tab. 2) was determined for number of leaves per plant (CV = 67.33%) and number of inflorescences per plant (CV = 61.02%). Both traits are highly dependent on plant age. Among the most uniform traits was floral diameter, which appeared to be relatively stable regardless of environmental conditions (CV = 13.89%). The highest variation (CV = 12.44%) was found for the Bizeljsko population, whereas the highest uniformity (CV = 9.88%) was established for Žumberak. The diameter of the largest flower, 13 cm, was in the Žumberak population. Another very important and relatively uniform trait was peduncle length (CV = 24.35%; Tab. 2). For cut flower production the most desired flowers are those peduncles at least 25 cm long. The highest variation of this trait was found for Bizeljsko (CV = 21.88%). The most uniform was the population in Logarska dolina (CV = 13.96%). The

TABLE 2. Statistical parameters for morphological description of the most important traits

Quantitative traits	No. plants	Mean	Min	Max	SD	CV (%)
No. of leaves per plant	1623	1.50	1.00	9.00	1.01	67.33
Petiole length	1603	19.28	5.50	37.00	4.37	22.66
Peduncle length	1665	17.04	7.50	37.00	4.14	24.29
Number of carpels	1560	6.21	2.00	12.00	1.28	20.61
Floral diameter	1666	8.35	4.50	13.00	1.16	13.89
No. of inflorescences per plant	1624	1.36	1.00	7.00	0.83	61.02
Qualitative traits	Percentages (total number of individuals = 1670)					
Pigmentation of leaf petiole	LPu (43.7); G (24.6); Pu (22.6); DPu (9.1)					
Color of sepals – abaxial side	Lpu, LLPu (27.8); W (26.4); Pu (17.2); GY, G (13.6); GYRe, GLRe, GRe, ReG, DGR, LReY, LReG, DReG (4.7); Re (3.4); DRe, DDR, RePu (2.0); DPu (2.0); WPi, WLPu, WPU (1.6); LLY, LY (1.3)					
Color of sepals – adaxial side	W (61.4); G (15.9); Pu (8.7); LLPu, LPu (4.0); DG, DDG, GYRe, GLRe, GRe, ReG, LLG, LRe (2.8); Re, DReG, DRe, DDR, RePu (2.1); YRe, ReY, GY, YG (1.8); WY, WPi, LLY, LY (1.8); Y, DY (1.5)					
Flower shape	wide shallow dish (66.6); dish (23.5); goblet (4.1); flat (3.9); narrow goblet (1.9)					

No. plants – number of analyzed plants; Min – minimum; Max – maximum; SD – standard deviation; CV – coefficient of variation; G – green; Pi – pink; Pu – purple; Re – red; W – white; Y – yellow; D – dark; DD – very dark; L – light; LL – very light; GRe – green-red (green predominant); DReG – dark red-green (red predominant); DDR – very dark red; GYRe – green-yellow (green predominant); GLR – green (predominant) combined with light red; LLPi – very light pink; WY – white-yellow; WLPu – white combined with light purple.

TABLE 3. Analysis of variance of the most important quantitative morphological traits used to differentiate the studied populations. Values represent means for studied traits \pm standard deviations

Population	N	No. of leaves	Petiole length	Peduncle length	No. of carpels	Floral diameter	No. of infloresc.
Bizeljsko	210	1.3 abfg* (± 0.8)	18.2 a (± 4.2)	14.8 a (± 3.2)	6.3 ab (± 1.3)	8.2 a (± 1.0)	1.1 ae (± 0.5)
Bohinjska bela	210	1.7 cd (± 1.3)	20.7 b (± 4.4)	15.0 a (± 2.7)	5.6 c (± 0.9)	7.9 b (± 0.9)	1.5 b (± 1.0)
Logarska dolina	210	1.4 bce (± 1.0)	19.4 c (± 4.0)	15.5 ab (± 2.2)	6.0 ac (± 1.1)	8.1 ab (± 0.8)	1.2 ac (± 0.8)
Sodražica	210	1.1 fg (± 0.4)	17.1 a (± 3.7)	15.9 b (± 3.3)	6.1 ad (± 1.2)	8.2 a (± 0.9)	1.1 a (± 0.3)
Črnomelj	199	1.1 efg (± 0.5)	17.3 a (± 3.4)	19.2 c (± 3.7)	6.2 aeg (± 1.1)	9.1 c (± 0.9)	1.1 a (± 0.4)
Žumberak	209	1.5 ac (± 0.9)	20.3 bc (± 3.9)	23.4 d (± 3.8)	7.1 f (± 1.5)	9.8 d (± 1.0)	1.3 ab (± 0.8)
Peca	210	1.5 ac (± 0.8)	18.2 a (± 3.4)	16.2 b (± 2.8)	6.5 be (± 1.3)	7.5 e (± 0.9)	2.2 d (± 1.0)
Rimske toplice	208	1.9 d (± 1.4)	23.1 d (± 4.2)	16.4 b (± 3.0)	5.8 cdg (± 1.1)	8.0 ab (± 1.0)	1.4 bce (± 0.9)
Total	1666	1.5 (± 1.0)	19.3 (± 4.4)	17.0 (± 4.1)	6.2 (± 1.3)	8.4 (± 1.2)	1.4 (± 0.8)
F		16.6	55.1	177.5	25.7	133.2	49.2
p		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

N – number of individuals; * – identical letters in columns indicate no significant difference based on Tukey's multiple-range test ($p < 0.05$).

plant with the longest peduncle (37 cm) was also from Žumberak.

Table 2 also reveals the predominance of plants with light purple and white abaxials (27.8% and 26.4%, respectively), sepals with a white adaxial side (61.4%), wide and shallow dish-shaped flowers (66.6%), and green or light purple peduncles.

PHENOTYPIC VARIATION IN DIFFERENT POPULATIONS

Analysis of variance indicated significant differences between the studied populations (Tabs. 3, 4). The Žumberak population was characterized by the longest

peduncles (23.4 cm \pm 3.8) and largest flowers (mean diameter 9.8 cm \pm 1.0). The plants in this population also had the highest average number of carpels. The inner part of all flowers (adaxial part of sepals) was green, whereas the outer (abaxial) part was green in 97.6% of the flowers. The dominant flower shape was a wide, shallow dish (85.2% of flowers). Flat flowers were also relatively common (9%). The high frequency of green flowers and the dominant wide, shallow dish shape can be considered an indication of late-stage flowering; flowers in early-stage flowering are whitish and have a goblet or narrow goblet shape.

TABLE 4. Differences between studied populations for various qualitative traits, and percentage of appearance of various phenotypes

Qualitative trait	Population	Percentages	
Pigmentation of leaf petiole	Bizeljsko	LPu (60.1); Pu (29.8); G (6.3); DPu (3.8)	ac*
	Boh. bela	LPu (41.3); Pu (31.7); DPu (16.4); G (10.6)	b
	Log. dolina	LPu (50.0); Pu (25.0); G (17.8); DPu (7.2)	a
	Sodražica	LPu (48.7); G (26.4); Pu (17.9); DPu (7.0)	c
	Črnomelj	LPu (50.2); G (36.4); Pu (12.4); DPu (1.0)	d
	Žumberak	G (65.6); LPu (21.5); Pu (12.4); DPu (0.5)	e
	Peca	G (33.3); LPu (28.6); Pu (23.3); DPu (14.8)	af
	Rim. toplice	LPu (47.3); Pu (27.5); DPu (19.9); G (5.3)	bf
Color of sepals, adaxial side	Bizeljsko	W (100)	a
	Boh. bela	W (90.5); Pu (4.6); LPu (2.9); LLY,LY (1.5); DPu (0.5)	b
	Log. dolina	W (92.4); Pu (3.3); LLY,LY (2.9); LPu (1.4)	bc
	Sodražica	W (90.9); LLY,LY (8.1); LPu (0.5); Pu (0.5)	bd
	Črnomelj	G (27.5); DG, DDG, GYRe, GLRe, GRe, ReG, LLG, LRe (22.0); YRe, ReY, GY, YG (14.5); other colours (36.0)	f
	Žumberak	G (100)	d
	Peca	Pu (60.0); LLPu, LPu (23.3); W (16.7)	e
	Rim. toplice	W (96.2); LLPu, LPu (1.9); WY, WPi, LLY, LY (1.4); Pu (0.5)	c
Color of sepals, abaxial side	Bizeljsko	LPu, LLPu (65.7); W (33.3); Pu (0.8)	a
	Boh. bela	LPu, LLPu (67.1); W (26.2); Pu (6.1)	b
	Log. dolina	LPu, LLPu (68.6); W (26.2); Pu (2.3)	c
	Sodražica	W (51.4); LPu, LLPu (39.5); Pu (0.8); LLY, LY (6.8); YRe, ReY, GY, YG (1.5)	acd
	Črnomelj	GYRe, GLRe, GRe, ReG, DGRe, LReY, LReG, DReG (37.5); Re (28.5); DRe, DDRe, RePu (17.0); G (4.5); other colors (12.0)	e
	Žumberak	G (97.6); GYRe, GLRe, GRe, ReG, DGRe, LReY, LReG, DReG (1.4); LPu, LLPu (1.0)	f
	Peca	Pu (70.0); W (14.3); LPu, LLPu (13.8); DPu (1.9)	g
	Rim. toplice	W (58.2); LPu, LLPu (18.6); Pu (12.3); WPi, WLPu, WPU (10.9)	cd
Flower shape	Bizeljsko	dish (55.7); wide shallow dish (30.0); goblet (8.1); narrow goblet (5.3); flat (0.9)	a
	Boh. bela	wide shallow dish (58.1); dish (36.2); goblet (4.3); flat (1.4)	bc
	Log. dolina	wide shallow dish (89.5); dish (6.3); goblet (1.4); flat (1.9); narrow goblet (0.9)	ab
	Sodražica	wide shallow dish (76.2); dish (11.4); flat (10.9); goblet (1.0); narrow goblet (0.5)	d
	Črnomelj	wide shallow dish (87.5); dish (10.5); flat (2.0);	ab
	Žumberak	wide shallow dish (85.2); flat (9.0); dish (5.2); narrow goblet (0.5)	de
	Peca	wide shallow dish (57.6); dish (34.8); goblet (7.6)	c
	Rim. toplice	wide shallow dish (49.5); dish (27.6); goblet (10.5); narrow goblet (8.1); flat (4.3)	be

Color symbols as in Table 2; *Identical letters in columns indicate no significant difference, based on chi-square test ($p < 0.05$).

The Peca population was characterized by the highest average number of inflorescences per plant (2.2 ± 1.0), and its floral diameter (average $7.5 \text{ cm} \pm 0.9$) was the lowest of all populations. The dominant floral shapes were dish (34.8) and wide, shallow dish (57.6%). The predominant floral color was purple or nearly purple.

The highest average number of leaves per plant (1.9 ± 1.4) was found in the Rimske toplice population. The leaves in this population also had the longest petioles.

PRINCIPAL COMPONENT ANALYSIS

When the data for quantitative variables were subjected to PCA, both principal components had eigen-

values above 1 (Tab. 5). PCA showed that 31.44% and 27.48% of the variation of the studied traits could be explained by the first and second principal components individually, and 58.92% together. Table 5 shows the weighting for the major traits explaining the maximum variation of each of the two principal components. Traits affecting the first principal component axis were peduncle length, floral diameter, number of carpels and petiole length. Traits representing populations along the second principal axis were number of leaves per plant and number of inflorescences per plant. The graph presenting the first principal component shows that the Žumberak population was distinguished from the others (Fig. 2). The scatter plots of the second principal component show high variation within populations. One consequence was that the studied popula-

TABLE 5. Eigenvalues, variation explained, and factor loadings for the analyzed quantitative traits

Principal component	1	2
Eigenvalues	1.89	1.65
Percentage variance	31.44	27.48
Percentage variance – cumulative	31.44	58.92
F	106.10	51.00
p	<0.01	<0.01
Number of leaves per plant	0.20	-0.84
Petiole length	0.50	-0.34
Peduncle length	0.83	0.18
Number of carpels	0.54	0.10
Floral diameter	0.77	0.30
Number of inflorescences per plant	0.12	-0.83

tions could not be clearly differentiated, although the mean values obviously differed.

DISCUSSION

The modern market requires diversity and continuous changes of cultivars. New cultivars can be obtained by direct selection of the desired genotypes from natural populations, or by selection of superior genotypes from progenies formed by artificial or natural hybridization. The cheapest and simplest way is direct selection from natural populations. Such selection can be successful only when natural populations are highly variable and when the needed traits are highly heritable. The variation ranges of some of the analyzed traits were found to be wider than those mentioned in the literature. Examples are floral diameter and number of carpels. The documented variation range of the first trait is 6–11 cm (Ravnik, 1969), but in our investigation was found to be 4.5–13 cm. The reported variation range of the second trait is 5–10 (Trinajstič, 1967; Frankel and Galun, 1977), but 2–12 in our study. Presently the most desired genotypes are those with long peduncles and large flowers. Profitable production requires high-yielding cultivars that develop 50 or more inflorescences per plant per season.

Our analysis of phenotypic variation indicates that the studied natural populations of Christmas rose contain valuable material which can be used to create new commercial varieties by direct selection from existing natural populations and/or by genetic recombination. It would be very useful to know the heritability of the studied traits, and whether the high variation is also associated with high heritability estimates and high genetic gain. The easiest and fastest way to obtain data about the heritability of the quantitative traits will be to make a series of crosses between phenotypically different individuals, create a sufficiently large offspring population, and calculate regression of off-

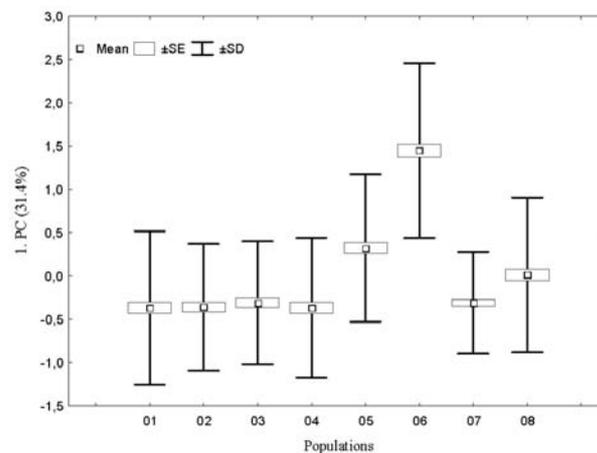


Fig. 2. Variability between naturally growing populations assessed by the first principal component. Populations numbered as follows: Bizeljsko (1), Bohinjska bela (2), Logarska dolina (3), Sodražica (4), Črnomelj (5), Žumberak (6), Peca (7), Rimske toplice (8).

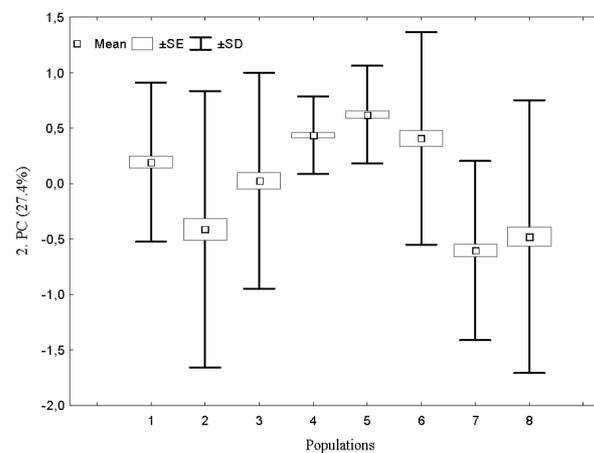


Fig. 3. Variability between populations assessed by the second principal component. Populations numbered as follows: Bizeljsko (1), Bohinjska bela (2), Logarska dolina (3), Sodražica (4), Črnomelj (5), Žumberak (6), Peca (7), Rimske toplice (8).

spring on one parent or on mid-parents (Falconer, 1981; Ollivier, 2002). The best way to determine environmental variance will be to study variation within clones (i.e., vegetatively propagated individuals of the same age) grown in different environments. Hybridization will probably result in genetically highly variable offspring populations, due to the predominant heterozygosity of the parental plants (the Christmas rose is predominantly a cross-fertilizing species; Hegi, 1975).

The Christmas rose is relatively new in genetic studies, and not much known about the inheritance of its traits. The number of inflorescences per plant is one

of the most complicated traits, and its expression depends on the genetic structure, plant age, environmental factors (which may have direct or indirect effects), and interactions which include genetic structure, plant age and environment. Plant age obviously has the greatest influence on the expression of this trait. Older plants have larger rhizomes and therefore more shoots. In our investigation, the highest number of inflorescences was recorded in the Peca population, located high in the mountains (1131 m a.s.l.). This may indicate that the expression of this trait also depends on the environment.

The next very important but less variable traits are peduncle length (CV = 24.29%) and floral diameter (CV = 13.89%). They were found to be highly influenced by the environment; in all studied locations, plants grown on deep and fertile soils developed longer peduncles than those grown in poor and shallow soils.

Traits associated with pigmentation (e.g., pigmentation of leaves, pigmentation of peduncles, floral color) present special problems. They are controlled genetically at least in part, but in some environmental conditions (e.g., under extreme sunlight or in deep shade) the genetic basis appears to become less important. In our case, the variation of pigmentation probably was due more to environmental factors (exposure to sunlight was probably crucial), although some populations were found to be characterized by darker pigmentation regardless of exposure to sunlight or shade (e.g., Žumberak population).

Statistical analyses of the quantitative and qualitative traits indicate that variability was expressed more within than between populations. This prevented clear differentiation between populations.

In terms of the present situation in the market, the most valuable populations are those containing plants with the longest peduncles and largest flowers. The highest values associated with those traits were recorded in Žumberak, which was found to be the most diverse population (Fig. 2). This finding is partly explained by its geographic location at 363 m a.s.l., south-east slope, and 1142 mm annual precipitation.

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