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Bioactive compounds produced by clones of *Rhodiola rosea* maintained in the Norwegian germplasm collection

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Roseroot, *Rhodiola rosea*, is a perennial herbaceous plant of the family *Crassulaceae*. The rhizomes of 95 roseroot clones in the Norwegian germplasm collection were analysed and quantified for their content of the bioactive compounds rosavin, salidroside, rosin, cinnamyl alcohol and tyrosol using HPLC analysis. All five bioactive compounds were detected in all 95 roseroot clones but in highly variable quantities. The ranges observed for the different compounds were for rosavin 2.90–85.95 mg g⁻¹, salidroside 0.03–12.85 mg g⁻¹, rosin 0.08–4.75 mg g⁻¹, tyrosol 0.04–2.15 mg g⁻¹ and cinnamyl alcohol 0.02–1.18 mg g⁻¹. The frequency distribution of the chemical content of each clone did not reflect a certain geographic region of origin or the gender of the plant. Significant correlations were found for the contents of several of these bioactive compounds in individual roseroot clones. A low, but not significant correlation was found between AFLP markers previously used to study the genetic diversity of the roseroot clones and their production of the chemical compounds. The maximum level of rosavin, rosin and salidroside observed were higher than for any roseroot plant previously reported in literature, and the roseroot clones characterized in this study might therefore prove to be of high pharmacological value.

1. Introduction

Roseroot, *Rhodiola rosea*, or arctic root also commonly known as golden root, is a perennial herbaceous plant of the family *Crassulaceae*. Roseroot is distributed from China into the mountain regions of Central and Northern Europe, in the coastal regions of North America as well as in Russia and in the Far East. Historically this plant has been described as an adaptogen (Kelly 2001), and Vikings used roseroot to enhance their physical strength and endurance (Magnusson 1992). In Russia, Northern Europe, China, and more recently in USA, roseroot has been used as a traditional herbal medicine, valued for its ability to enhance human resistance to stress or fatigue (Sanderberg and Bohlin 1993; Darbinyan et al. 2000). It promotes longevity and has been claimed to have antiallergenic, anti-depression and anti-inflammatory effects, to result in enhanced mental alertness and give cardio-protection, and has been used for a variety of therapeutic applications including cancer therapy (Kurkin and Zapesochayaya 1986; Maslov et al. 1997; Razina et al. 2000; Spasov et al. 2000; De Bock et al. 2004; Mattioli and Perfumi 2007).

The chemical composition of roseroot has been widely studied (Dubichev et al. 1991; Kurkin et al. 1986; Furmanowa et al. 1999; Ming et al. 2005; Galambosi et al. 2007). The active metabolite salidroside and its aglycon tyrosol (Kuryanov et al. 1991; Antipenko and Kuznetsov 1998) and cinnamic glycosides such as rosin, rosavin, and rosarin (Zapesochayaya and Kurkin 1982; Kurkin et al. 1986; Satsyperova et al. 1993) have been well characterized. Other constituents of *Rhodiola* roots are flavonoids, tannins and gallic acid and its esters.

Investigations on the phytochemistry of roseroot have revealed the presence of about 28 compounds classified into six distinct groups (Brown et al. 2002). The main biologically active compounds are salidroside, tyrosol, cinnamyl alcohol glycosides, rosavin, rosarin and rosin (Kucinskaite et al. 2007; Wiedenfeld et al. 2007). There are nearly 200 *Rhodiola* species but cinnamyl alcohol glycosides and monoterpenoid, rosiridol and its glucoside rosiridin have been found only in *R. rosea* (Ganzera et al. 2001). It has previously been shown that the total flavonoid content of the Nordic plants ranged from 0.34 to 0.51%, while those of German and Austrian origin had a content of 0.12–0.29% (Galambosi et al. 1999).

HPLC has been used to characterize and quantify the chemical composition in several plants including *Rhodiola* species. Globally, more than 60% of the total pharmaceutical market is derived from plants (Wakdikar 2004). The composition and contents of chemical compounds are consequently one of the most important traits in a commercial breeding program of such plants and a grouping of accessions in corresponding germplasm collections based on their chemical composition would be useful. Clones with high chemical content may carry genes that can be used to improve *R. rosea* collections. So far natural wild resources of *Rhodiola* species have been harvested and used as raw material for the pharmaceutical market. The natural growth of *Rhodiola* species is mostly limited to quite specific areas and in other countries it has been shown that human disturbance for commercial purposes have reduced their genetic diversity (Yan et al. 2003; Lei et al. 2006; Xia et al. 2007). One possible solution could be the establishment of *ex situ* and *in situ* conservation of these plants to ensure the production of safe,

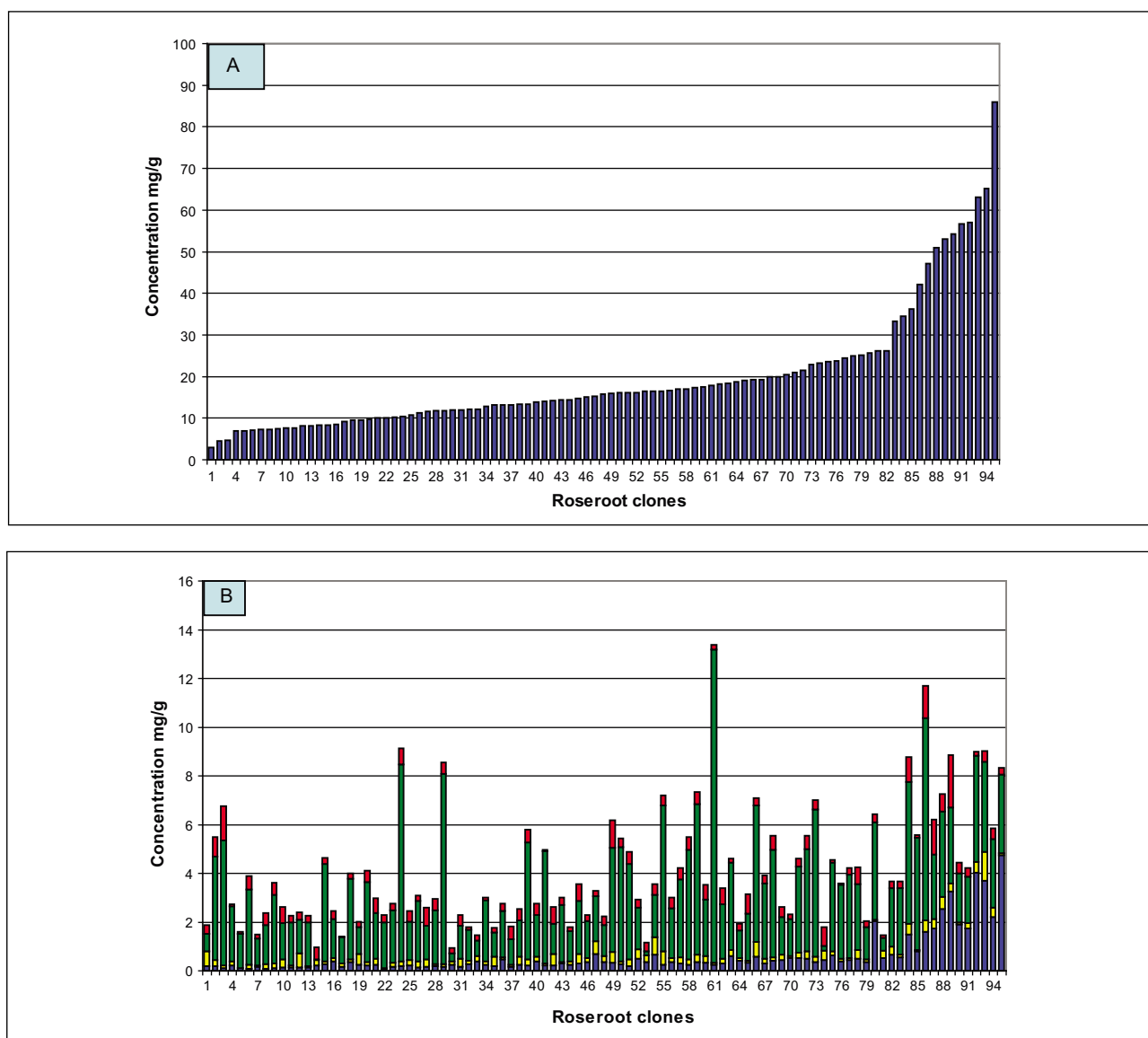


Fig. 1: Quantities of five bioactive compounds produced by Norwegian roseroot determined by HPLC. All quantities are measured in mg g^{-1} . A) The content of rosin in 95 roseroot clones sorted based on increasing levels of rosin. B) The content of tyrosol (red), salidroside (green), cinnamyl alcohol (yellow) and rosin (blue). The roseroot clones are listed in the same order as in A

efficient and stable pharmaceutical products. In Norway a roseroot germplasm collection has been established and vegetative cultivation of this plant has been successfully introduced (Dragland 2001; Elameen et al. 2008).

The aims of this study were to: (i) identify and measure the production of the bioactive compounds rosin, salidroside, rosin, cinnamyl alcohol and tyrosol in 95 roseroot clones originating from 15 different counties in Norway but grown under uniform experimental conditions for three growing seasons; (ii) compare the chemical content of the Norwegian roseroot collection with

corresponding results from roseroot in other countries, and (iii) investigate if genotypes producing high levels of bioactive compounds are genetically related and whether AFLP markers can be used to identify genetic resources of roseroot for further development in future breeding programmes. A breeding programme in Norway followed by roseroot farming will be necessary in order to counteract the threat of human disturbance for commercial purposes that otherwise might result in a reduction or extinction of genetic diversity of natural roseroot populations.

Table 1: Quantities of the main bioactive compounds of roseroot

Compound	Minimum level	Maximum level
Rosavin	2.900 ± 0.006	85.950 ± 0.710
Salidroside	0.030 ± 0.001	12.850 ± 0.001
Rosin	0.080 ± 0.001	4.750 ± 0.001
Cinnamyl alcohol	0.020 ± 0.001	1.180 ± 0.002
Tyrosol	0.040 ± 0.001	2.150 ± 0.002

Values are presented in $\text{mg g}^{-1} \pm$ standard deviation

2. Investigation and results

The chromatographic fingerprints from HPLC showed large qualitative and quantitative differences of the chemical constituents in the *R. rosea* clones included in this study. The five chemicals rosin, salidroside, rosin, cinnamyl alcohol and tyrosol, were detected in all 95 roseroot clones, but high variation between the quantities of these compounds were revealed among the clones in the germplasm collection (Fig. 1A and 1B). Single roseroot clones with high production of chemical compounds did not reflect a certain county of origin or plant gender. Minimum and maximum levels of these bioactive compounds

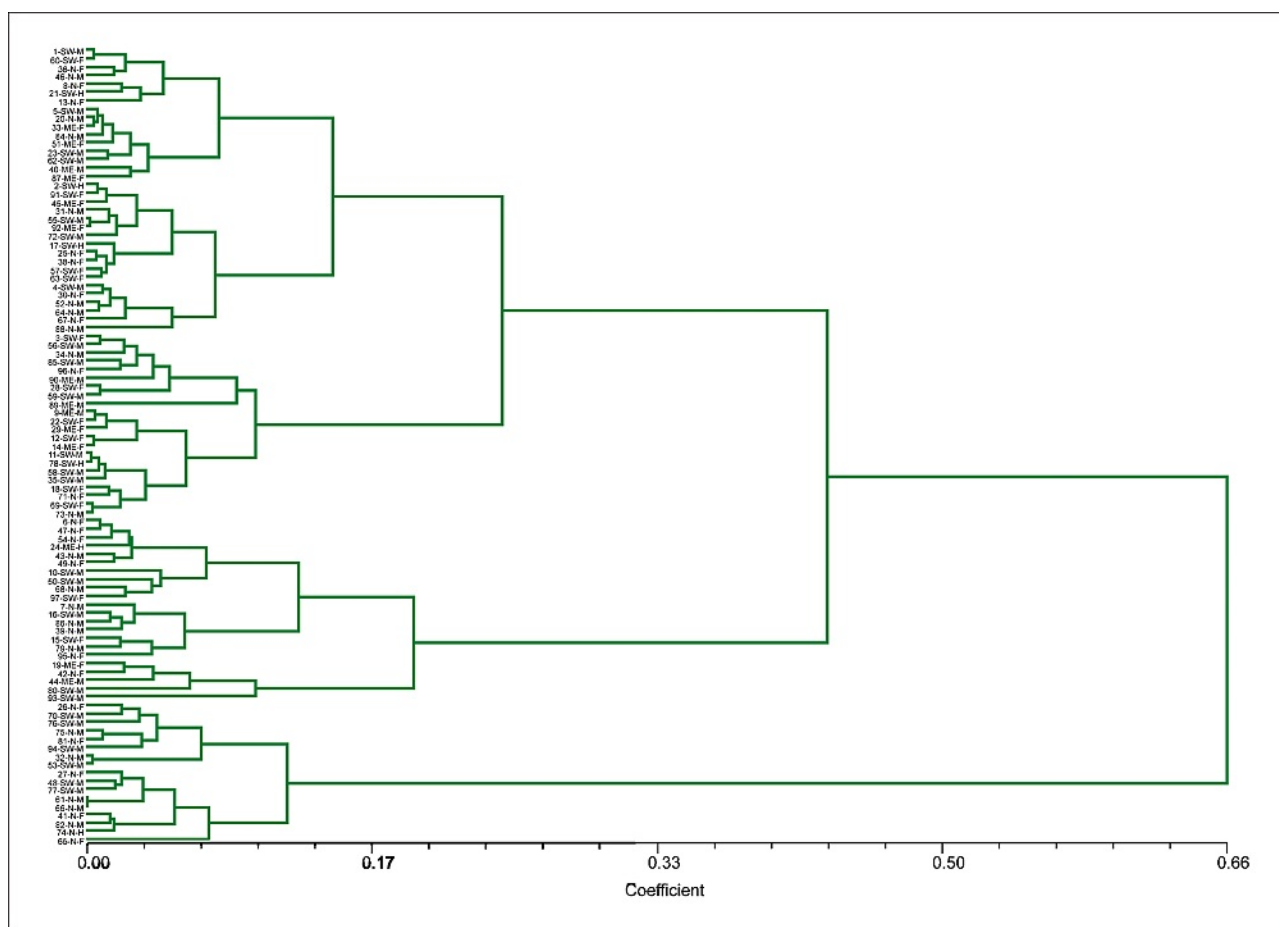


Fig. 2: The relationships between the Norwegian roseroot clones determined by UPGMA cluster analysis based on their chemical production. Each roseroot clone was labelled according to the code given by Elameen et al. (2008)

measured in the Norwegian roseroot clones were recorded (Table 1).

The relationships between the 95 clones revealed by UPGMA cluster analyses based on chemical production data are presented in Fig. 2. The UPGMA result did not reflect the county of origin of the clones.

Pearson correlation analysis showed significant and positive correlations of the content of several of these bioactive compounds produced in the roseroot clones analysed (Table 2). Best correlation was found for the production of the important bioactive compounds rosavin and salidroside ($r=0.6194$; $P \leq 0.01$). A negative correlation was found between salidroside and rosin. For some of the individual Norwegian clones the level of chemical compounds exceeded the level required for roseroot to be used for clinical treatment (Table 3).

Regression analysis showed a low, but not significant correlation between the results from a previously performed AFLP analysis (Elameen et al. 2008) and the chemical composition of the roseroot clones. The respective R^2 values were 16.140,

14.650, 5.480, 10.440 and 11.891 for rosavin, salidroside, rosin, cinnamyl alcohol and tyrosol.

3. Discussion

In this study we describe the chemical characterization of a collection of Norwegian roseroot clones. As far as we know this represents the largest collection of vegetatively propagated roseroot plants.

Except for cinnamyl alcohol, the maximum levels of the bioactive compounds presented in this study were much higher than levels of bioactive compounds found in other *Rhodiola* species (Wang et al. 2006; Mao et al. 2007; Wiedenfeld et al. 2007).

Differences in the content and composition of the essential chemical compounds of roseroot have been reported in several publications (e.g., Galambosi et al. 1999; Linh et al. 2000; Ganzera et al. 2001; Wiedenfeld et al. 2007). The chemical compositions of the extracts produced from roseroot rhizomes

Table 2: Pearson correlation coefficients (r) for the correlation analysis between the contents of bioactive compounds produced by 95 individual roseroot clones

	Rosavin	Salidroside	Rosin	Cinnamyl alcohol	Tyrosol
Rosavin	1.0000				
Salidroside	0.6194**	1.00000			
Rosin	0.2774*	-0.0339	1.0000		
Cinnamyl alcohol	0.16676	0.0783	0.4587**	1.0000	
Tyrosol	0.2163*	0.2253*	0.2819*	0.2060*	1.0000

* Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

Table 3: Quantities of the different chemical compounds produced by roseroot reported in the literature

Origin of plants	Literature reference	Rosavin	Salidroside	Rosin	Cinnamyl alcohol	Tyrosol
Norway	This study	85.950	12.850	4.750	1.180	2.150
China	Linh et al. (2000)	ns	11.100	ns	ns	2.200
China	Ma et al. (2008)	0.650	11.140	3.580	ns	1.120
Finland	Makarov et al. (2003)	0.790	0.280	0.120	0.080	ns
Finland	Galambosi et al. (2009)	18.140	7.380	ns	ns	ns
Lithuania	Kucinskaite et al. (2007)	3.688	1.352	1.603	ns	ns
Mongolia	Wiedenfeld et al. (2006)	18.700	13.100	ns	18.900	ns
Poland	Wiedenfeld et al. (2006)	27.900	4.000	ns	10.500	ns
Russia	Kurkin et al. (1986)	25.000	12.000	ns	ns	ns
Russia	Kurkin et al. (1988)	ns	ns	1.000	ns	ns
Russia	Makarov et al. (2003)	4.110	0.930	0.530	0.300	ns
Russia	Kucinskaite et al. (2007)	0.562	1.624	2.574	ns	ns
Sweden	Wiedenfeld et al. (2006)	50.700	0.000	ns	15.600	ns
USA	Ganzera et al. (2001)	3.500	2.700	0.800	ns	ns

ns = not studied

Values given are in mg g⁻¹ or mg ml⁻¹. The highest level of the five bioactive compounds detected in the studies is listed

in Norway were found to be highly variable. This high variation observed might be due to the large number of clones investigated here. When the maximum levels of the five main chemical compounds in the roseroot clones included in this study were compared to maximum levels observed in roseroot sampled from other countries (Table 3) (Kurkin et al. 1986; Kurkin et al. 1988; Linh et al. 2000; Ganzera et al. 2001; Makarov et al. 2003; Kucinskaite et al. 2007; Wiedenfeld et al. 2007; Ma et al. 2008), we found that some Norwegian roseroot clones had a very high production of rosavin, salidroside and rosin. However, cinnamyl alcohol was found to be lower than what was found in most other studies. Yield of plants used for the pharmaceutical market must be defined in terms of the production of specific bioactive compounds. Roseroot extracts used in most clinical studies have been standardized to contain 0.8-1% salidroside and a minimum of 3% rosavin (State Pharmacopoeia USSR 1990; Brown et al. 2002). Surprisingly the compound salidroside was absent from a roseroot sampled from the Swedish health market (Wiedenfeld et al. 2007), and the levels of salidroside and rosavin from the two samples collected from the Russian health market were below these requirement levels (Makarov et al. 2003). Interestingly, when comparing our results with previous studies, individual clones from Norway are the only clones that meet the chemical requirements of a product to be used for clinical treatments (Table 3).

The chemical compositions in plants are mainly influenced by genetic factors (Coleman and Jones 1991; Hashimoto and Yamada 1994) and a mutation in a single gene might affect the production of certain compounds (Vogel et al. 1996). Also environmental factors like climatic conditions, day length, growth place, fertilization and the stage of the plant development will affect the chemical composition (Vogel et al. 1999; Mihalov et al. 2000). In this study the plants had been grown on the same field under exactly the same environmental conditions for three growing seasons. Consequently the differences observed in chemical compounds produced can only be explained by genetic factors. The results presented here clearly show that genetic diversity among individuals of roseroot clones in Norway influence their chemical profile. The significant correlation between the production of the bioactive compounds rosavin and salidroside showed that Norwegian roseroot clones might have important medicinal and pharmacological values. The chemodiversity found in this study provides the opportunity to select naturally occurring superior clones, and the technology developed facilitates the clonal propagation of these individuals to create novel, consistent plant germplasm. The observed low levels of cin-

nemyl alcohol suggest that it will be a potential topic for a future breeding program to increase the concentration of this compound in Norwegian roseroot.

Single clones of roseroot with high production of chemical compounds do not reflect a certain gender or region of origin. This result supports the hypothesis of high gene flow of these clones, in agreement with a recent study where these same clones were characterized for their genetic diversity (Elameen et al. 2008). The chemical analysis indicated that there is a heterogeneity within and among these *R. rosea* clones of different countries. This supports the hypothesis that the production of the chemical compounds is determined by the genetic characteristics of these clones and that the chemical profile could not be attributed to certain environmental conditions where the plants were collected or grown. This was different from previous reports where the essential chemical compositions of roseroot have been found to vary according to the geographic origin of the plant (Galambosi et al. 1999; Kucinskaite et al. 2007; Wiedenfeld et al. 2007). Only little correlation was found between the results from our previous AFLP analysis (Elameen et al. 2008) and the chemical composition of the roseroot clones. This might be due to the fact that the polymorphism detected by AFLP is limited to non-coding regions of the roseroot genome.

Roseroot is distributed all over Norway, from the south to the north and at different altitudes (Skottsberg 1928; Dragland and Mordal 2006). The frequency distribution of the chemical content of each clone did not reflect a certain geographic region of origin or the gender of the clones. It is clear that genetically heterogenic clones of roseroot can be collected within any county regardless to their geographic origin. This will enable commercial farming of roseroot at different geographic regions in Norway, an activity that will reduce the exploitation of wild roseroot for commercial purposes and assist to maintain its genetic diversity.

The high variability of the quantity and quality of the chemical compositions observed in this study and the high genetic diversity which were recently observed in this germplasm (Elameen et al. 2008) need to be considered when assessing traits such as chemical compositions for breeding programs. This is the first report that combines the analysis of genetic diversity with information of the chemical composition of roseroot. Further studies of the roseroot populations from Norway as well as from other countries should be performed throughout the following years to identify clones with optimal chemical composition and to maintain high genetic diversity of this species.

4. Experimental

4.1. Plant materials

Ninety five single wild growing plants of *R. rosea* were collected from fifteen different counties in Norway representing 65 municipalities in Norway (Dragland and Mordal 2006). These plants were vegetatively propagated, planted and grown under uniform experimental conditions at a field at the Norwegian Institute for Agricultural and Environmental Research (Bioforsk Øst Apelsvoll). After three growing seasons of vegetative growth, 50 g were collected from the rhizomes of each roseroot clone, dried at 70 °C and sent to Interregional Centre 'adaptogen' St. Petersburg, Russia for HPLC analysis. The genetic diversity of these clones has recently been characterized (Elameen et al. 2008).

4.2. HPLC analyses

One gram of ground sample was extracted with 10 ml of methanol in a boiling bath. The extract was separated by reverse phase chromatography on a Beckman System Gold L HPLC with a Luna C18 column (150 × 4.6 mm, 5 µm) and the compounds detected with a UV detector at wavelengths 254 and 280 nm. A linear gradient of acetonitrile – water with 0.05% trifluoroacetic acid (10-100% acetonitrile for 90 min) was used. The flow rate was set at 1.0 ml min⁻¹ and the injection volume was 20 µl. These analyses were performed at the Interregional Centre "Adaptogen", St. Petersburg, Russia.

4.3. Characterization of chemical compounds

The compounds rosavin, salidroside, rosin, cinnamyl alcohol and tyrosol were identified by the comparison of their retention index values and UV spectral characteristics against the corresponding information for standards used (Makarov et al. 2003). The concentrations of the compounds were recalculated based on absolute dry raw plant weight as described by Makarov et al. (2003).

4.4. Clustering analysis

A data matrix of the 95 clones and the five chemical compounds (rosavin, salidroside, rosin, cinnamyl alcohol and tyrosol) was constructed. The data matrix was standardized and analyzed to estimate relationships among the clones which were obtained using the Manhattan coefficient. Final dendrogram was constructed with the un-weighted pair-group method with arithmetic mean (UPGMA) analysis. The analyses were done using NT SYS-pc version 2.2 (Rolf 2001).

4.5. Correlation analysis

Pearson correlation coefficients were calculated to assess correlations between the production of the five bioactive compounds rosavin, salidroside, rosin, cinnamyl alcohol and tyrosol in the roseroot clones included in this study. The correlation analysis was done using PROC CORR of SAS program (SAS, 2002-2003).

Association between AFLP markers and the production of bioactive compounds of roseroot clones was estimated through stepwise multiple regression analysis, where each chemical compound was treated as a dependent variable while the AFLP marker was treated as an independent variable as described by Virk et al. (1996). To select independent variables for the regression equation, F-values with 0.045 and 0.099 probability were used to enter and remove, respectively as suggested by Roy and Bargmann (1957), and Affifi and Clark (1984). The analysis was done using MINITAB program.

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