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Short communication

GENETIC DIVERSITY OF WHITE CLOVER (*Trifolium repens* L.) FROM THE URBAN AREA OF RĪGA

Dace Grauda[#], Kalvis Avotiņš, Oksana Fokina, Agnese Kolodinska-Brantestam, and Isaak Rashal

Institute of Biology, University of Latvia, Miera iela 3, Salaspils, LV-2169, LATVIA

[#] Corresponding author, dace@email.lubi.edu.lv

Contributed by Isaak Rashal

*White clover (*Trifolium repens *L.) is well represented in the urban environment and wide areas. The goal of this study was to compare the genetic features and to determine the genetic distances and similarities between some populations of white clover using iPBS (inter primer binding sites) technique in a retrotransposon-based method in samples from ecologically different areas in Latvia. Comparisons were made between three plant groups: urban environment (different areas in Rīga), Latvian countryside territory (four different localities), and the commercial variety 'Daile'. The Shannon diversity index was not high (0.2974 for Rīga, 0.3079 in countryside territories, and 0.3367 for the variety 'Daile'), but the polymorphic bands present in all evaluated plant sets were higher than 89%. Using principal coordinates analysis the white clover formed three clusters. One cluster included plants from the variety 'Daile'. The plants from Rīga urban area and different sites of Latvia formed two clusters.*

Key words: *white clover, urban environment, genetic diversity, iPBS method.*

White (Dutch) clover *Trifolium repens* L. is a cross-pollinated perennial herb that belongs to the legume family (Leguminosae Juss.), *typicum* variety, both diploid (2n = 2x = 16) and tetraploid plants (2n = 4x = 32) are known (Pētersone and Birkmane, 1980; Zohary, 1984; Holms, 1992; Voisey *et al*., 1994; Uva, 1997; Jansone, 2008). White clover occurs worldwide and is one of the plant species cultivated in the temperate climate zone in meadows, yards, gardens, along roads and streets etc. (Roze, 2003; Roze, 2007; Ravagnani *et al*., 2012). It is also one of components in grasslands of the capital of Latvia, Rīga. Due to the fact that white clover is widespread in urban areas, including areas with various environmental pollution levels, it is a perfect plant species for adaptation studies (Mather, 2000). The analysis of genetic diversity is expected to show diversity among plants of the same population and differences among populations. This knowledge is one of the basics factors to clarify the population development in changing environmental conditions and under anthropogenic load (Biemont *et al*., 2006). In studies of urban ecology, where multifactor interactions are studied, for population genetic investigations it is important to use a rather simple method, which gives the possibility to analyze a sufficiently large number of samples and different species (Otto, 2006; Collins, 2012).

A universal retrotransposon-based method iPBS (inter primer binding sites), which was developed by Kalendar *et al.* (2010) allows to show high levels of genetic diversity and it is cost and labour effective (Kumar *et al*., 1999; Bennetzen, 2000; Kumar *et al*., 2001; Agarwal, 2008). It has been successfully used for investigation of genetic diversity for several species (SanMiguel *et al*., 1996; Semagn *et al*., 2006; Schulman, 2007; Vukich *et al*., 2009; Kalendar *et al*., 2010; Smykal *et al*., 2011; Kļaviņa *et al*., 2014). The aim of this study was to compare the genetic diversity between Latvian urban (Rīga) and wild country-side populations of white (Dutch) clover.

To perform genetic diversity analysis, white clover plants fresh leafs were collected and desiccated in silica gel. Leaves of 37 plants from five different urban areas in Rīga (Rumbula, Sarkandaugava, Šķirotava, Bastejkalns, and Bolderāja) and 26 plants from each of five places in Latvia (Augstkalne; Kurzeme, Stikli; Katleši; Nīca; Rucava) were collected in June and July 2014. The distances between the harvesting areas were more than 200 km, with one exception — distance between the Nīca and Rucava sites was about 30 km. The distances between collected wild white clover plants were more than 1 m to eliminate the possibility that plants are from a single gamete (Khanlou *et al*., 2011). Also, 23 seeds of the variety 'Daile' that were used in greening in Rīga were used in this study.

DNA from dried leaves was extracted using the NucleoSpin Plant II kit (Macherey-Nagel, mn-net.com) and standard protocol for plants. After homogenisation of dried plant material lysis buffer PL2 was used (protocol part 2B). Incubation time was 60 min. Extracted DNA was examined for quality by 1.7% agarose gel electrophoresis at 80 V for one hour followed by detection with a spectrophotometer. Eleven iPBS primers (Kalendar *et al*., 2010; Kalendar and Schulman, 2014) that showed high level of polymorphism in genomes of many organisms were tested on a preliminary sample set of white

clover, which represented in several localities. Two primers, 2076 (GCTCCGATGCCA), 2079 (AGGTGGGCGCCA), were selected for screening of all plant material in this study. The repeatability of each of selected primers was tested with duplication of 10 top polymorphic bands produced from samples. 64 bands were detected by primer 2076 and 43 bands by primer 2079. In total, 107 bands were analyzed; all of them were polymorphic (Shannon's Diversity index 0.3504). DNA amplification was performed in a Gene Amp® PCR System 9700 thermocycler (Applied Biosystems) under the following conditions: denaturation 95 °C/3 min, then 30 cycles (denaturation 95 °C/45 s, primer annealing 50 min and 4 °C storing). 2 µl of DNA was used for one reaction. PCR mixture total volume was 25 µl and consisted of 17.225 µl dd H_2O, 2.5 µl 10× Dream Taq Buffer (Fermentas), 0.5 µl of 10mM dNTPs (Fermentas), 2.5 µl primer, and 0.25µl of 5 u/µl *DreamTaq* polymerase (Fermentas), and 2.5 u/µl of 0.025 *Pfu* DNA polymerase (Fermentas). PCR products were visualised by 1.7% agarose gel electrophoresis with 25 × 10 gel track, in 1XTAE buffer at 40 V for 17 hours. Gel was dyed for 40 minutes in deionised water with 20 µl/1 L 10% water solution of ethidium bromide and rinsed for 10 minutes in deionised water. After visualisation the agarose gel was documented with a digital camera (Yilmaz *et al.*, 2012). The amplified fragments were scored as presence (1) or absence (0) of a band of a given length in bp. Data were evaluated using POPGENE version 1.32 and NTSYSpc 2.1 software.

White clover is a cross-pollinated species with diploid (2n = 2x = 16) and tetraploid (2n = 4x = 32) plants, and consequently the genetic diversity within species and populations potentially should be high (Williams, 1987; Voisey *et al.*, 1994; Ellison *et al.*, 2006; Zhang *et al.*, 2007). Although the polymorphism level between individual white clover plants was high (Table 1) the number of polymorphic bands in all investigated groups was about 90. The Shannon diversity index (*I*), which shows the internal genetic diversity of plant groups, was not high: 0.2974 in Rīga, 0.3079 in countryside sites, and 0.3367 for the variety 'Daile'. In studies of genetic diversity of three varieties from UK, Netherlands, and Denmark (Khanlou *et al.*, 2011), Shannon diversity (*I*) was higher (0.487, 0.459 and 0.421, respectively), although the number of polymorphic loci was significantly lower (22–51 depending on used primer). This indicates that the great number of polymorphic loci or bands in the plant sets determine a small number of plants. The breeding varieties of cross-pollinated species are a complex of genetically different plants with similar phenotype, which can likely explain why the Shannon index of the studied wild plant sets and the commercial variety 'Daile' did not significantly differ.

Genetic distances (Nei, 1978) between the studied groups were small: between white clover from the urban area of Rīga and countryside sites of Latvia it was 0.0124, between the Rīga plant group and variety 'Daile' — 0.0382 and between the countryside plant group and the variety 'Daile' — 0.0407.

In a principal coordinates analysis the white clover plants clustered into three groups (Fig. 1). One group included plants from variety 'Daile'. The plants from Rīga urban area and countryside sites of Latvia were separated in two groups: one contained plant samples from Rumbula (Rīga) and Augstkal-

Table 1

NUMBER OF POLYMORPHIC BANDS OF DIFFERENT SAMPLE SETS

Sample set	The number of polymorphic bands	Shannon index
Rīga	91	0.2975
Different sites of Latvia	89	0.3079
Variety 'Daile'	91	0.3367

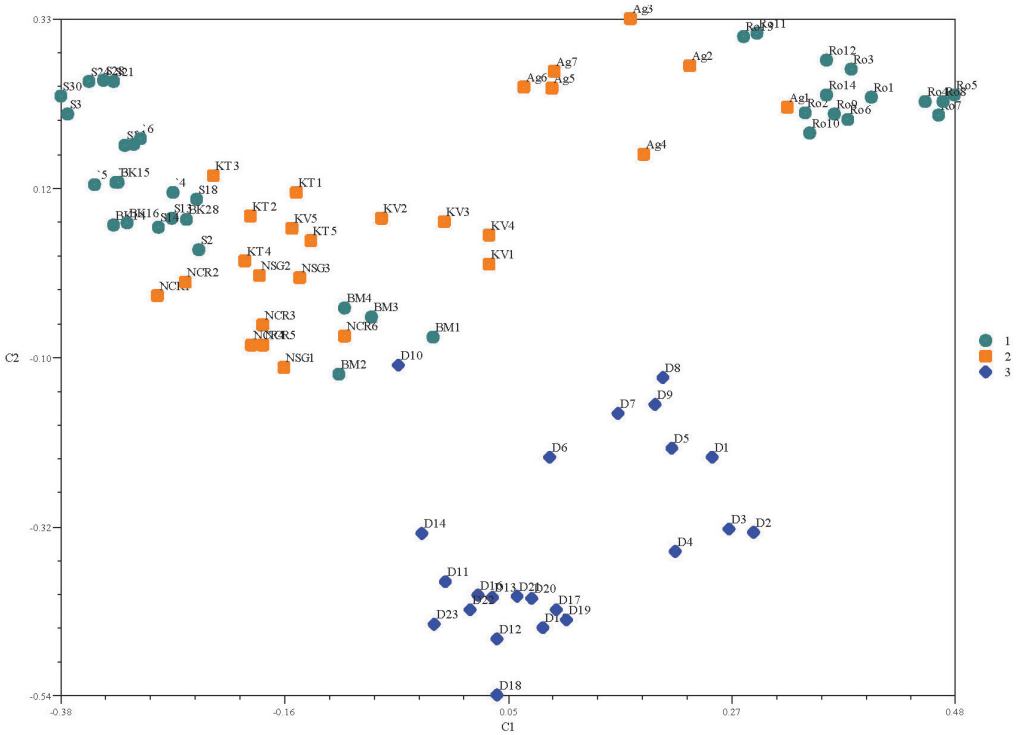

Fig. 1. Principal coordinates analysis of white clover population based on genetic similarity. 1, white clover plant group from different sites of Rīga (Ro, Rumbula; S, Sarkandaugava; Š, Šķirotava; BK, Bastejkalns; BM, Bolderāja), 2, white clover plant group from different regions of Latvia (Ag, Augstkalne; Kv, Kurzeme, Stikli; KT, Katleši; NSG and NRC, Nīca, Rucava), 3, white clover plant set from plants of variety 'Daile'.

ne, and the other contained remaining plants. It seems likely that the same variety was used in agricultural areas of Augstkalne and in street greenery of Rīga.

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BALTĀ ĀBOLIŅA (*Trifolium repens* L.) ĢENĒTISKĀ DAUDZVEIDĪBA RĪGAS PILSĒTVIDĒ

Baltais āboliņš (*Trifolium repens* L.) ir tauriņziežu dzimtas augs, plaši izplatīts gan pilsētvidē, gan ārpus tās. Tas tiek izmantots kā lauksaimniecības augs biomasas iegūšanai, un tā ir arī populāra daudzgadīgos zālienus veidojošā suga — pilsētvidē balto āboliņu sēj zālājos. Baltais āboliņš plaši pārstāvēts dažādos biotopos ar mainīgu ārējo faktoru ietekmi, tai skaitā pilsētvidē. Tādēļ tas ir piemērots modeļobjekts antropogēnās ietekmes pētīšanai. Darba mērķis bija noteikt baltā āboliņa Latvijas populācijas ģenētisko daudzveidību, izmantojot iPBS (*inter primer binding sites*) — uz retrotranspazoniem balstītu metodi. Iegūtie rezultāti salīdzināti starp trim augu paraugkopām: pilsētvidē augoši augi (Rīgas pilsētas teritorija), dabiskā vide (Latvijas novadu teritorijas) un selekcionētā šķirne 'Daile'. Lai gan polimorfo lokusu skaits visās pētītajās grupās bija augsts (89%), tomēr Šanona (*Shannon*) indekss, kas raksturo grupu iekšējo ģenētisko daudzveidību, pētāmajām grupām svārstījās tikai ap 0.3. Šāds rezultāts norāda uz to, ka iekšējo grupu polimorfismu nosaka neliels ģenētiski atšķirīgu augu skaits. Galveno koordinātu analīzē, kas balstīta uz Šanona indeksu, visi pētītie paraugi izveidoja trīs grupas. Vienā no tiem grupējās šķirnes 'Daile' augi, savukārt baltais āboliņš no pilsētvides un Latvijas reģioniem izveidoja divas atšķirīgas grupas.