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Genetic diversity of red clover determined by morphological traits and SSR molecular markers

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Genetička divergentnost crvene deteline determinisane morfološkim osobinama i *SSR* molekularnim markerima

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Abstract

Red clover (*Trifolium pratense* L), is one of the most important forage crops in tropics and grown widely under diverse environmental conditions. It remains as the most reliable source of animal food. The genetics of various yield attributing and qualitative traits in red clover remains poorly understood because of the heterozygous nature of the crop.

Knowledge on genetic diversity of any crop usually helps the breeder in choosing desirable parents for use in their breeding program. The diverse genotypes or accessions can be crossed to produce superior high yielding hybrids possessing resistance to various abiotic and biotic stresses. In the present scenario, there is an urgent need to evaluate the available red clover accessions for the extent of genetic diversity. Molecular markers are powerful tools, which help in differentiating plant varieties at the DNA level and have been widely used for genetic diversity studies in a number of crop species.

Among the various molecular markers, the SSR markers are reliable due to their specific nature and its importance for the assessment of genetic diversity owing to their speed and simplicity. Understanding the molecular diversity of available clones of *Trifolium pretense* L will be helpful in widening the genetic base of available red clover clones for use in breeding programs. In the present investigation, 40 red clover genotypes from 17 different countries around the world obtained from Institute of Field and Vegetable Crops, Novi Sad were screened for their genetic diversity using 13 morphological traits and 15 SSR markers. Morphological traits were recorded in the germplasm raised during 2011 sown the experiment was laid out in a randomized complete block design with three replications

Analysis of variance for the quantitative traits revealed that differences among accessions were significant for all traits. This indicated that morphological traits differed in amount of variation between red clover accessions studied, According to the correlations values of internodes had the strongest genetic correlation with number of branch (r = 0.95**) and Medium lamina diameter was in lowest correlation and non-significant with number of branch (r = 0.25^{ns}). Principal component analysis (PCA) of the quantitative data was performed to investigate the importance of different traits.

Applied the GGE biplot model accounted for 83.7% of the total variation of the standardized data, consisting of 69.2 and 14.5 % of variance attributable to PC1 and PC2 respectively.

Based on the diversity of morphological traits, the red clover accessions were grouped into 6 clusters with genetic distances ranging from 0.56 to 10.35. Therefore, the phenotypic markers provide a useful measure of genetic distances among red clover accessions to identify potential donors or parental.

In this study UPOV data for morphological descriptors, across the red clover accessions studied the genetic similarity index was ranged from 0.00 to 1.00.Through SSR analysis, a total of 1146 alleles were generated the number of fragments amplified by each SSR primer combination varied from 24 for RCS3681 to 108 for RCS1729 with an average value of 76.4 per primer combination. The similarity coefficients based on 15 SSR markers ranged from 0.50 to 0.90 among the 40 accessions studied.

The clustering of genotypes was performed using UPGMA method. The dendrogram constructed using SSR markers the dendrogram produced ten distinct clusters. The cluster size varied from 14 genotyps of red clover (cluster 1) to 1 (Clusters 9, 10)

According to Principal coordinates analysis the first (PCoA 1) and the second (PCoA 2) principal coordinate axes accounted for 5.5% and 5% of the total variation, respectively. Analysis of molecular variance (AMOVA) using SSR data according level of polyploidy revealed that the total variation observed was high and accounted for 99.91% between groups and 0.09% was observed among groups.

Key words: Red clover, morphological traits, SSR marks.

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Sažetak

Crvena detelina (*Trifolium pratense* L), predstavlja jednu od najvažnijih krmnih biljaka u tropima, koja se gaji u različitim uslovima sredine i čini glavni izvor stočne hrane. Genetika komponenti prinosa i kvalitativnih osobina crvene deteline nije dovoljno proučena zbog heterozigotne prirode vrste.

Poznavanje genetičkog diverziteta bilo koje kulturne vrste pomaže oplemenjivaču pri izboru roditelja za ukrštanje u okviru oplemenjivačkog programa. Različiti genotipovi i linije se ukrštaju da bi se dobili superiorni hibridi sa otpornošću na abiotički i biotički stres. Postoji suštinska potreba da se postojeći genotipovi crvene deteline procene za nivo genetičkog diverziteta. Molekularni markeri predstavljaju moćnu metodu koja diferencira biljne varijetete na DNK nivou i korišćena je u brojnim studijama genetičkog diverziteta brojnih kulturnih vrsta.

SSR markeri od svih ostalih molekularnih markera pokazuju visoku pouzdanost u studijama genetičkog diverziteta zbog specifične prirode i jednostavnosti izvođenja metode. Razumevanje molekularnog diverziteta dostupnih klonova *Trifolium pratense* L. će pomoći u proširivanju genetičke baze klonova crvene deteline za korišćenje u oplemenjivačkim programima. U ovom istraživanju 40 genotipova crvene deteline iz 17 različitih država sveta, dobijene iz Instituta za ratarstvo i povrtsrastvo, Novi Sad, je ispitano za nivo genetičkog diverziteta korišćenjem 13 morfoloških osobina i 15 SSR markera. Morfološke osobine su merene u eksperimentalnom ogledu izvedenom 2011 godine po potpuno sličajnom blok sistemu sa tri ponavljanja.

Analiza varijanse je pokazala da su razlike između vrednosti kvantitativnih osobina bile značajne za sve osobine. Ovo je ukazalo na to da su i morfološke osobine dobar pokazatelj nivoa varijabilnosti genotipova crvene deteline. Broj internodija je bio genetički korelisan sa brojem ogranaka (r = 0,95**), dok je korelacija dijametra centralne lamine i broja ogranaka je bio neznačajan (r = $0,25^{ns}$). Analiza osnovnih komponenti (PCA) je primenjena za kvantitativne osobine da bi se utvrdila značajnost različitih osobina.

Primenjeni GGE biplot model je objasnio 83,7% ukupne varijanse standardizovanih podataka, pri tom je PC1 komponenta imala 69,2, a PC2 komponenta 14,5 % ukupne varijanse. Na osnovu diverziteta morfoloških osobina, genotipovi crvene deteline su grupisani u 6 klastera, dok se opseg genetičkih distanci kretao od 0,56 do 10,35. Stoga fenotipski markeri takođe predstavljaju dobar pokazatelj genetičke distance između genotipova crvene deteline, i mogu da pomognu u identifikovanju potencijalnih roditelja i donora određenih osobina.

Na osnovu UPOV podataka morfoloških deskriptora crvene deteline, izračunat je indeks genetičke sličnosti koji je bio u opsegu 0,00-1, 00. SSR analiza je utvrdila ukupno 1146 alela dobijenih amplifikacijom SSR prajmera, i najmanji broj je bio 24 za prajmer RCS3681 a najveći 108 za prajmer RCS1729. Prosečan broj alela je iznosio 76,4 po kombinaciji prajmera. Koeficijenti sličnosti dobijeni na osnovu 15 SSR markera su se kretali u opsegu 0,50 -0,90 u okviru 40 proučavanih genotipova.

Klasteri genotipova dobijeni primenom UPGMA metoda su pokazali postojanje 10 odvojenih klastera. Veličina klastera je varirala od jednog genotipa (klasteri 9 i 10) do 14 genotipova (klaster 1).

Na osnovu analize glavnih koordinata (PCoA)-prva (PCoA 1) i druga (PCoA 2) osa su sadržavale 5,5% i 5% ukupne varijanse. Analiza molekularne varijanse (AMOVA) na osnovu SSR podataka i nivoa poliploidije je otkrila visoku ukupnu varijansu koja je iznosila 99,91% između grupa i 0,09% u okviru grupa.

Ključne reči : Crvena detelina, morfološke osobine, SSR markeri

UDK broj: 633 32:575.113.832(043.3).

List of abbreviations

AA Homozygous

Aa Heterozygous

AFLP Amplified fragment length polymorphism

AMOVA Analysis of molecular variance

ANOVA Analysis of variance

CRD Completely randomized design

CTAB Cetytriammonium bromide

DOH Density of hairs

DNA Deoxyribonucleic acid

GD Genetic distance

GHA Growth habit

IWM Intensity of white marks

ISSR Interspersed simple sequence repeats

LCO Leaf color

LSD Least significant difference

MAS Marker-assisted selection

MLD Medium lamina diameter

MLL Medium lamina length

N Nitrogen

NOB Number of braches

NOI Number of internodes

PCA Principal Component analysis

PCR Polymerase chain reaction

PCoA Principal coordinate analysis

RGA Resistance gene analogs

PGR Plant genetic resource

RAPD Random amplified polymorphic DNA

RFLP Restriction fragment length polymorphism

QTL Quantitative trait loci

SNP Single nucleotide polymorphism

SSR Simple sequence repeat

STD Stem thickness

STL Stem length

STS Sequence tagged sites

TFL Time of flowering

UPOV International Union for the protection of new varieties of plants

UPGMA: Unweighted pair-group method using arithmetic averages

UV Ultraviolet

YDM Yield of dry matter

YGM Yield of green mass

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1. INTRODUCTION

Red clover (*Trifolium pratense* L.) is a perennial forage legume of limited persistence, mainly used for cutting in grass-clover leys 2-4 years duration , but also occurring nature in permanent grassland it is one of the most cultivated forage legumes in the world (Dias et al., 2008).

In comparison to alfalfa (*Medicago sativa* L.), red clover ranks second but still higher than white clover (*Trifolium repens L.*).

Red clover can be grown in pure stand but is best mixed tall growing grasses, such as Italian or hybrid ryegrass (*Lolium multiflorum lam*. or *Lolium boucheeanum kunth*) under moist and winter mild conditions, timothy *(Phleum pretense* L.) or meadow fescue (*Fescue pratensis Hbds*.) under harsher conditions. Admixture of a grass increases yield and yield stability, prevents from nitrate leaching and enhances nitrogen fixation efficiency (Nyfeler et al., 2009).

Red clover can also use as a starter legume to facilitate establishment of withe clover in complex mixture for long term grassland mixture (Suter et al., 2008).

It has excellent seedling vigor, and is a great pasture protein source. Seedling of red clover form a purely vegetative primary shoot, with leaf carrying an axillary bud capable of forming lateral shoots which have the potential of becoming generative stems, which have a limited number of extended internodes (4-6) and terminate with a flower head.

Red clover is an herbaceous (non-woody), short lived perennial plant. Stems develop from the crowns that mostly growing at or slightly above soil level, but cultivars vary in this regard, stem lengths at maturity may vary up to 80cm in length. Stems are hollow and pubescent (hairy). Growth habit varies from erect to prostrate.

Leaves are arranged alternately on the stems. The first true leaf is unifoliate with succeeding leaves being trifoliate (three hairy leaflets per petiole). Individual leaflets are usually marked with a whitish V. However, leaves of some plants may have no mark (Bowley et al., 1984b).

Flower heads, located at the tip of stems, usually have 75-125 individual pinkish-violet flowers.

Red clover seeds are mitten shaped, 2-3 mm long and vary in color from yellow to brown to purple. The top develops from a pronounced taproot which penetrates very deeply and possesses an extensive system of laterals.

Figure1a.Red clover leaf

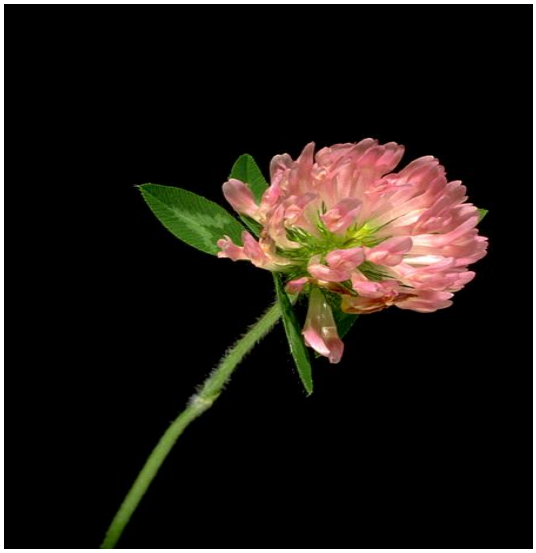

Figure1b. Red clover inflorescence

Figure1c.Red clover florets

Figure1d.Red clover seeds

The root system is dominated by a strong tap root developing from primary seedling root. The tap roots will normally die and disintegrate in the second year and the plants will depend on secondary roots for their survival (Montpetit and Coulman, 1991a). Root depth is intermediate between that of shallow rooting white clover and that of deep rooting alfalfa

Soil pH decreases caused by unreasonable agricultural measures presented in a few years, rainfall, leaching, organic matter decay, and harvest of crops. Optimum pH for plant growth varies with crop species and with soil physical and chemical properties (Lanyon and Griffith, 1988).

Soil pH influenced available Mn, Al, and P levels. Most growth and fewer diseases problems occur when the soil pH between 6.4-6.8 (Peaslee and Tylor, 1989). Legume yield response under different levels of soil acidity has not been well quantified, and small-seeded legumes differ in their relative tolerance or resistance to low soil pH (Voigt and Mosjidis, 2002).

Red clover is one of the most important legumes in the world because of its adaptation to a wide range of soil types, pH levels between 6.0 - 7.5 environmental and management conditions (Taylor and Queensberry, 1996).

Red Clover and other legumes, with the help of *Bacteria Rhizobia* are able to fix nitrogen gas from the atmosphere and convert it into a form of nitrogen that plants can utilize. When it comes to forage growth nitrogen is very limiting factor. As natural gas prices rise so does nitrogen costs. This makes legumes an even more important aspect of a good forage program. With the rise of fertilizer costs many cattle producers are looking into incorporating legumes into their system. Up to 400 kg N ha-1 per year (Carlsson and Huss-Danell, 2003).

In contrast to the great importance of red clover, as the economy and the agro ecological aspect, it is evident that this reduction in area under forage legumes in the world. Historically a drastic reduction in area under clover (red and white clover) came after in 1909. The German chemist Fritz Haber developed the process of extraction of nitrogen through ammonia synthesis, so that by World War II, the industrial production of nitrogen has increased significantly, and its application in the production of mineral fertilizers, and low prices of these fertilizers have led to a decline in area under the clover, which is significantly reduced their participation in rotation (Taylor, 2008).

Red clover is an important perennial forage legume because it is used as hay or as pasture in crop rotations and has high productivity and the rapid regeneration after harvest and excellent quality forage (Murray et al., 2007). It is especially suitable for cattle breeding, because it has quality and nutritive hay (Acikgoz, 2001).

Most researchers report that the optimal stage for red clover cutting both in terms of yield and quality is when about 20-25% of the flowers appear. At this stage, dry matter digestibility ranges between 65 and 70%, and after that it declines (Wiersma et al., 1998).

The growth habit of red clover varies from erect to prostrate, with numerous leafy stems arising from the crown each year. The large leaves are usually carry a horseshoe shaped mark and divided into three leaflets. The undersides of the leaves are hairy. Heads consist of approximately 125 flowers. Flowers are predominantly self-sterile and require pollination by bees for seed set. The pods are short and single seeded.

Red clover contains large amounts of provitamin A and vitamins C, D, E, K, B1, B2 and B3. It is also rich in minerals (Markovic et al., 2007). It is exceeds corn and oat grains in the content of essential amino acids (cystine, tryptophan, leucine, Vasiljevic et al., 2009). These qualities have made red clover useful for hay, silage, pasture, intercropping and green manure in several countries.

It is an outcrossing species, with a diploid genome (2n = 2X = 14, Sato, 2005). With a gametophytes self-incompatibility system (Taylor and Quesenberry, 1996), however tetrapod (2n=4x=28) type is known (Jansone, 2008). The red clover populations are heterogeneous and showed high levels of genetic variation within and among populations (Vasiljevic et al., 2000).

Red clover is cross-pollinated specie with a gametophytic self incompatibility system insects, for example honey bee (*Apis* spp), bumblebees *(Bombus* spp.) are required for flower pollination, in addition it is heavily relied on to pollinate a wide variety of crops (Delaplane and Mayer, 2000), thus red clover populations are heterogeneous and of heterozygous individuals.

Generally, red clover varieties differ in quality according to ploidy level and earliness. A decrease of protein content from early to late populations was reported by (Makarenko and Pribytkov, 1989). Mineral contents of forages are very important for animal feeding. A number of inorganic elements are essential for normal growth and reproduction of animals. The tetraploid red clover is characterized by a higher content of protein, potassium and phosphorus and by a lower content of fiber than diploid red clover (Bieniaszewski and Fordoński 1996). Concentrations of N, Ca, Mg, Fe and Co, pectin and lignin are generally higher than in grasses but other constituents are similar or lower. Mineral contents, particularly P, K, Ca, Mg, Cu and Zn, are increased by early spring application of the growth regulators daminozide and mefluidide. Republic of Serbia is an exceptionally rich source of natural autochthonous genetic resources of forage crops (Tomic, 1997). Family *Fabacea,* which contains major forage culture in Serbian flora, has 34 genera, the most important are: *Trifolium, Vicia, Medicago* and *Lotus,* whereas in family Poaceae there are 70 genera.

Red clover is the second most important perennial forage legume in Republic of Serbia, where it is planted on about 120.000 ha. The domestic market is predominated by cultivars developed in domestic research centers (Institute for Forage Crops, Kruševac, and Institute of Field and Vegetable Crops, Novi Sad).Characteristically, legume seeds have large protein contents, ranging from 20% to as much as 40% of their dry matter, according to species, genotypes within species, and environments breeding of perennial legumes based on genetic methods had started at the beginning of the 20th century (Lamb et al., 2006). In Serbia, it started relatively late alfalfa in Novi Sad in 1947, few years later in Forage Crops Institute in Kruševac (Mijatovic and Ranković, 1975). Work on development of red clover cultivars started at Kruševac Institute (Miladinović, 1972), and much later at Novi Sad Institute (Vasiljević et al., 2005). The domestic diploid cultivars of red clover were synthetics composed of native genotypes or local populations. Starting from 1960 and using different breeding methods, 10 red clover cultivars were developed, nine diploid and one tetraploid. The main objectives of red clover breeding had been high production potential for yield of biomass, adaptability to the production conditions of the mountain-hill region of central Serbia, especially its slightly acid soils, as well as development of genetic structures genotypes tolerant to economically important diseases and capable of bringing high yields of quality forage (Vasiljević et al., 2005 a). To be successful in breeding work, it was important to correctly select breeding targets and methods as well as to gather breeding material with as high genetic variability as possible, the latest generation of domestic red clover cultivars was developed using methods such as mass and phenotypic recurrent selection, (Lugić et al., 2001a. Lugić et al., 2001b).

2.OBJECTIVES AND AMIS

The Serbian core collection with 40 accessions of red clover, an important forage species, is little described. The overall objective of this study was to analyze, evaluate and describe the magnitude of genetic diversity of a set of cultivars and populations from the core collection at the morphological and molecular level.The aim to extract some valuable cultivars and populations of red clover. Thirteen different quantitative and qualitative morphological traits, collected in the experimental field of the Institute of Field and vegetable Crops (Rimski Šančevi) near Novi Sad.

Fourteen SSR markers were used to describe 40 cultivars and populations from Institute's core collection cultivated in Serbia. We are going to describe morphological and molecular variation between cultivars. Cluster analysis based on the morphological traits and molecular marker traits will reveal different distinct clusters that separated the cultivars according to their traits. Use of genetic diversity in breeding programs requires using the most promising cultivars and populations of red clover, to combine positive traits such as forage yield, and probably to use within population variation to detect valuable genotypes that could be used as parents of synthetic varieties.

We are going to choose the best genotypes for parent's combination for further selection of red clover and getting good offspring.

3. LITERATURE REVIEW

Grasslands, including steppe, grassy savanna, prairie, and shrub savanna tundra, cover approximately 40 % of the global land, besides their importance for production forage for domestic livestock (White et al., 2000).

Forage legumes are richness in protein, vitamins, and mineral matter. As roughage, they are also characterized by increased fiber content over 18% crude cellulose (Grubic and Adamovic, 2003).

It is offer the potential to increase intake and feeding value of pasture, to lift animal production, to complement seasonal growth of grasses, and to improve product quality (Cosgrove et al., 2003; Hutton et al., 2011; Steinshamn, 2010). They also fix atmospheric nitrogen (N) in the soil and reduce parasite loads in ruminants (Abberton et al., 2006; Marley et al., 2005).

Many researchers have indicated that one tone of biomass dry legumes (aboveground and undergrounds) can approximately bind 30 – 40 kg of nitrogen from the air this is the one of the main nitrogen sources in organic farms. Perennial legume grasses overtake grain legumes by nitrogen fixation from the atmosphere, increase productivity of crop rotation plants and improve soil properties (Weston et al., 2002).

Compared to grasses, clovers in general have higher nutritive value such as more crude protein, digestibility, minerals, and vitamins. When mixed with grasses such as tall fescue (*Festuca arundinacea* Schreb), they can reduce entophyte toxicity (Lacefield and Ball, 2000).

Red clover (*Trifolium pratense* L.) has important medicinal properties due to the presence of antioxidants (Atkinson et al., 2004). And it is one of the main forage species from temperate regions originated from Eurasia where wild-type populations are found in the Caucasus Mountains. In its natural habitat it is found in meadows, forest margins, and field borders (Herrmann et al., 2006).

Red clover is also grown less extensively in other temperate regions of the world such as Japan, South Africa, Argentina, Columbia, Mexico, Chile, New Zealand, and Australia (Taylor and Queensberry, 1996) and recently naturalized in North America (Bou et al., 2003). Its domestication and introduction into European farming systems dates back to the sixteenth century and first concerned Spain, northern Italy, and some regions of northwest Europe.

Molecular evidence (Herrmann et al.,2005), has recently showed a clear separation between local landraces and spontaneous of red clover from permanent meadows in Switzerland, while the group of landraces was difficult to separate from traditional and advanced cultivars of any European origin .

Red clover will adapt best to areas where summer is moderately cool or warm with best growth when the temperatures are between 21 to 24°C and moisture is adequate throughout the growing season (Taylor, 1985).

Traditionally, the legume has been devided into three families: *Caeasalpinieaceae, Mimosoidaceae,* and *Fabaceae*.

Fabaceae, group most of the economically important food and feed legume i.e *Medicago, Lens, Lythyrus, Vicia, Cicer, Glycin, Vigna* (Doyle and Luckow, 2003).

The *Fabaceae,* also called *Leguminosae,* is the third largest family of flowering plants with more than 36 tribes, 727 genera and 19,327 described species, in terms of agricultural and economic importance. Legumes includes a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fiber, fuel, fertilizers, timber, medicinals, chemicals, and horticultural varieties . Taxonomically Fabaceae has been traditionally divided into three subfamilies: *Mimosoideae, Caesalpinioideae* and *Papilionoidea*e based on characteristics particularly of the flower.

Red clover and several other important crops such as soybean (*Glycine max*) or pea (*Pisum sativum*) as well as the model legume *Lotus japonicus* all belong to the same subfamily Faboideae, but to different tribes (Choi et al., 2004).

According to classification of Zohary and Heller (1984), red clover is placed in section *Trifolume* . And is selected as the type species (lectotype) of the genus *Trifolium* L.The most closely related genus is *Melilotus* (sweet clovers) and the genus *Medicago,* including alfalfa and other medic species, is also part of the *Trifolieae*. As a consequence, M. *truncatula* shares a very recent common ancestor with alfalfa and a relatively recent ancestor with the clovers and sweet clovers. Translational genomics based on whole genome sequencing of *M. truncatula* (Young et al., 2005; Zhu et al., 2005).

Kingdom **Plantae**

Division Briophyta **Spermatophyta** Pteridophyta

Subdivision Gymnospermae **Angiospermae**

Class Monocotyledoneae **Dicotyledoneae**

Order Capparale s **Fabales** Rosales

Family Quillajaceae Polygalaceae **Fabaceae** Surianaceae

Subfamily Mimosoideae **Faboideae** Caesalpinioideae

Tribe Phaseoleae Loteae **Trifolieae** Viceae

Genus *Medicago* ***Trifolium***

Section *Lotoidea* e.g. *Trifolium repens* ***Trifolium***

Species *T. diffusum* ***T. pratense*** *T. medium*

Variety ***T. p. var. sativum*** ***T. p. var. pratense*** *T. p. var. nivale*.

Figure 2: Taxonomic relationship of the family of *Fabaceae*. Key taxa and species mentioned in these are listed (Baltisberger, 1995; Choi et al., 2004; Zohary and Heller, 1984).

The genus name for clover derives from the Latin tres, "three", and folium,"leaf," so called from the characteristic from of the leaf, which has three leaflets (trifoliate) *Trifolium* consists of about 230 species annual and perennial species including red some of them extensively cultivated as forage and green manure (Repková et al., 2006).

Red Clover (*Trifolium pratense* L.) and other species of agricultural importance, such as white clover (*Trifolium repens*), have a wide distribution and adaptation to different agro ecological regions (Ellison et al., 2006; Vižintin et al., 2006), although, only a few of them are commercially used as forage crops

Morphological characterization is the first step in description and classification of genetic resources. Performance of a plant in field will determine whether it would be chosen in a breeding program. Phenotypic variances that are basically resulted from interaction between environment variance and genotypic variance should have correlations with genetic diversity in the molecular level.

The breeding program on forage legumes has two main objectives, namely, basic research and development of new cultivars for use as forage and cover crops. The more basic research objectives are: 1) to identify and evaluate physiological, morphological and molecular traits that can be used as selection criteria in developing germplasm of forages: 2) to study population genetics, genetic structure and genotype-environment interactions to formulate selection strategies conducive to the development of improved germplasm through conventional and unconventional breeding techniques.

High forage yield of good quality is primary breeding targets in plant breeding forage of good quality includes biomass, yield production, persistence, as well as high content of crude protein contributing to a well-balanced and high-class feed of cattle. Red clover is a most popular fodder legume suitable for growing in agro-ecological conditions, and is used as summer- and winter-forage for cattle (Jansone, 2008).

The main task for red clover breeders is to create new varieties, suitable for local circumstances, with high yield, disease- and pest-resistance, good wintering qualities. Overall when various sets of cultivars or populations of red clover were analyzed, large genetic diversity were determined among and within populations using morphological traits and molecular markers (Greene et al., 2004; Dias et al., 2008).

Morphological characteristics are the strongest determinants of the agronomic value and taxonomic of plants (Riday and Brummer, 2004). Based on morphological characters alone, it is difficult to distinguish accessions of red clover from each other because they have overlapping variations in terms of the major delimiting morphological and biological characters such as stem length, stem thickness, number of internodes, length and width of central lamina of medial leaflet, shape of medial leaflet, color of leaf in the year of sowing, intensity of white marks on the leaf time of flowering, growth habit, yield of green mass per plant, yield of dry matter (Vasiljevic et al., 2007) .

Diverse taxonomic characteristics have been used to separate and assess patterns of phenotypic diversity in the relationships of species and germplasm collections of crops (Perry and MacIntosh, 1991; Rabbani et al., 1998).

Genetic diversity in crop species can be determined by using the agro-morphological as well as biochemical and molecular markers (Geleta et al., 2007, 2008).

It is the first step in description and classification of genetic resources (Smith and Smith, 1989), the valuable characteristics of red clover (productivity, resistance to biotic and non-biotic factors, as well as quality) depend on a series of morphological and physiological traits which, in turn, are influenced by genetic and environmental factors, in this respect

various sets of cultivars or populations of red clover were analysed, large genetic diversity were determined among and within populations using morphological traits and molecular markers (Greene et al., 2004; Dias et al., 2008).

Traditionally, diversity is estimated by measuring variation in phenotypic, qualitative traits or quantitative agronomic traits such as yield potential, stress tolerance (Kameswara, 2004).

Therefore molecular variability studies can help in the utilization and conservation of germplasm, as they provide a background for the development of better strategies for germplasm maintenance.

Biological diversity' means the variability among living organisms from all sources including, among other things, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems. Some more details a definition given by (FAO and IUFRO, 2002).

Biological diversity covers the number, variety and arrangement of living organisms (i.e. all life on earth). Diversity at various levels may substantially influence composition functioning productivity and sustainability of managed and non-managed ecosystems (vellend and Geber, 2005). The distribution of biological diversity has spatial or temporal patterns, or whether there is a relationship between the amount of diversity and the level of primer productivity in an ecosystem (Prosser et al., 2007). Study on genetic diversity is critical to success in plant breeding, it provides specific information about breeding objectives, and identifies parental combinations exploitable to create segregating progenies with maximum genetic potential for further selection, (Liu et al., 2000 ; Dje et al., 2000 ; Aremu et al., 2007). Diversity measures have been extensively developed and have been instrumental in understanding fundamental ecology properties of many types of communities. They are the subject of recent reviews both for their general application (Koleff et al., 2003; Magurran, 2004).

Genetic diversity can be measured at many different levels, including population, species, community, and biome. Which level is used depends upon what is being examined and why, but genetic diversity is important at each of these levels, the amount of diversity at the genetic level is important because it represents the raw material for evolution and adaptation. More genetic diversity in a species or population means a greater ability for some of the individuals in it to adapt to changes in the environment. Less diversity leads to

uniformity. Within species, genetic diversity often increases with environmental variability. Species richness is widely used in ecology as a measure of species diversity.

For the improvement of diversity in a breeding program wild populations as well as landraces may be a source for traits such as resistance to biotic or abiotic stresses, forage quality or male sterility and can be integrated in breeding programs for the targeted improvement of a specific trait (Rao et al., 2003).

Genetic diversity among individuals or populations can be determined using morphological and molecular markers. Phenotypic characters have limitations since they are influenced by environmental factors and the developmental stage of the plant, In contrast, molecular markers, based on DNA sequence polymorphisms, are independent of environmental conditions and show higher levels of polymorphism.

Genetic diversity is an essential raw material for evolution, refers to the variation of heritable characteristics present among alleles of genes in different individuals of populations of species. It is refers to sum total of genetic variations found in a species or population (Singh, 2001).

Landraces play a more prominent for red clover breeding than in other forage species. This is due to the cultivation history of the species and to the relatively strong differentiation between cultivated and wild (spontaneous) forms. Molecular marker has also been used to identify eco geographic races within the domesticated or wild gene pools of crop species (Liviero et al., 2002; Yu et al., 2003).

Genetic markers are specific chromosomal fragments within a genome, which presence and absence can be monitored, respectively. They allow for a rapid assessment of genetic diversity on the genome level and have been widely used to characterize genetic resources in various plant species (Beebe et al. 2001; Kubik et al., 1999).

The genetic diversity in the germplasm of a breeding programme affects the potential genetic gain through selection. Estimates of genetic diversity using new molecular tools, especially molecular markers have proven to be a useful way to delineate existing heterotic groups, identify new heterotic groups and assign inbreds of unknown genetic origin to established heterotic groups (Dubreuil et al., 1996; Hongtrakul et al., 1997; Saghai-Maroof et al., 1997; Pejic et al., 1998; Casa et al., 2002).

The introduction of DNA-based markers during the second half of 20th century has changed the pace and precision of genetic analysis (Dodgson, et al., 1997). During the last two decades DNA-based markers have led to the construction of whole genome linkage maps in many plant and animal genomes, a crucial step for several downstream applications

such as gene cloning, genome analyses and marker-assisted selection of agricultural crops (Cullis, 2002; Paterson, 1996a).

All living organisms are known to be made up of cells that are programmed by genetic material called DNA, in a diploid individual (i.e. where chromosomes are organized in pairs), there are two alleles of every gene – one from each parent (Ruan and Sonnino, 2007).

The concept of genetic markers is not a new one; Gregor Mendel used phenotype-based genetic markers in his experiment in the nineteenth century. The limitations of phenotype based genetic markers led to the development of more general and useful direct DNA based markers that became known as molecular markers it is offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues molecular markers may or may not correlate with phenotypic expression of a trait.The markers are typically small regions of DNA, often showing sequence polymorphism in different individuals within a species and transmitted by the simple Mendelian laws of inheritance from one generation to the next.

The use of molecular markers allows the direct assessment of genotypic variation at the DNA level. As genes involved in traits of agronomic importance are mapped and tagged, markers assist in breeding programs and even to isolate the gene. Marker analysis helps to understand the genetic make-up of the accessions and also make it possible to analyze the global organization of genetic diversity within a species. Several statistical techniques are available for the analysis of genetic diversity using DNA fingerprinting data. Ultimately the phenotypic variations observed can be correlated to the molecular marker profile.

There are different marker systems available such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), sequence tagged sites (STS), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), single nucleotide polymorphism (SNP), Interspersed simple sequence repeats (ISSR) and other gene derived markers like resistance gene analogs- DNA (RGA-DNA) markers. molecular marker technique should have the following criteria: be polymorphic and evenly distributed throughout the genome, provide adequate resolution of genetic differences, generate multiple, independent and reliable markers, simple, quick and inexpensive, need small amounts of tissue and DNA samples, have linkage to distinct phenotypes and , require no prior information about the genome of an organism. Molecular markers have wide application in different branches of life sciences.

In genetics, molecular genetic markers are defined as differences at the genotype level that can be used to answer and explain questions of genetics (Lokko et al., 2005).To be useful as a genetic marker, of DNA sequence with a known location on a chromosome and associated with a particular gene or trait, the marker locus has to show experimentally detectable variation among individuals (Sorensen et al., 2008).

Molecular markers are now widely used to track loci and genome regions in several crop-breeding programmers, as molecular markers tightly linked with a large number of agronomic and disease resistance traits are available in major crop species (Phillips and Vasil, 2001; Jain et al., 2002, Gupta and Varshney, 2004).

In recent years molecular markers have become available in both animal and plant systems for basic and applied studies, increased knowledge and ability to characterize genetic diversity in the germplasm pool for essentially crop species. And provided basic information about genetic diversity before and after domestication from its wild ancestor, among geographically distributed landraces, and within historically elite germplasm (Cooper et al., 2004; Niebur et al., 2004; Buckler et al., 2006).

It is widely accepted that microsatellites (simple sequence repeat) are one of the most informative markers have been broadly applied to various areas of genetic research including studies of genome variation, genetic mapping, determination of evolutionary relationships and comparative genome analyses (Zhenlin et al., 2005).

Thanks to the increasing knowledge of the genome of different organisms (animal and plants) it is possible to exploit the sequence information to design specific primers for amplification of specific loci throughout the entire genome. An example of this application is represented by simple sequence repeat (SSR) markers, they consist of di-, tri- or tetra-nucleotide motifs repeated several times [for instance, (CA) n, (GAT) n or (AGCT) n] which are a common feature of most eukaryotic genomes.

Their high level of polymorphism, combined with their high interspersion rate, makes them an abundant source of genetic markers (Wu and Tanksley 1993; Plashke et al., 1995; Roder et al., 1995).

SSR markers have an unprecedented ability to generate highly informative data for examining genetic diversity, population structure, and evolution (Korzun, 2003).

In comparison with other molecular marker techniques, highly polymorphic and informative, technically simple, quick, reproducible and relatively inexpensive when primer information is available, and efficient technique. It has high reproducibility. The use of radioactivity is not essential. Therefore widely used in plants in genetic research, cultivar characterization,

plant breeding, paternity tests and phylogenetic studies among others (Real et al., 2007; Dias et al., 2008; Santos et al., 2010; Zhang et al., 2010).

The primers are not proprietary (as in SSR) and can be synthesized by anyone. Variations in primer length, motif and anchor are possible. The primers are long (16–25 bp) resulting in higher stringency. Furthermore, SSR markers often occur in gene-rich genome regions, increasing their potential relevance for allele-trait the amplified products (SSR markers) are usually 200–2000 bp long and amenable to detection by both agarose and polyacrylamide gel electrophoresis.

Microsatellites have been developed in different crop plants and currently the most frequently used. In genetic diversity studies, popular markers in livestock genetic characterization studies (Sunnucks, 2001).

These features make microsatellites powerful genetic markers for genome (genetic, physical, comparative and association) mapping, genetic diversity, marker-assisted diagnosis / selection, population and evolutionary studies in eukaryotic species including mammals, plants, and insects.

Microsatellites commonly used to assess genetic relationships between populations and individuals through the estimation of genetic distances (Beja-Pereira et al., 2003; Ibeagha- Awemu et al., 2004; Joshi et al., 2004).

They have also been identified in plant chloroplasts as well as in plant and animal mitochondrial genomes (van Oppen et al., 2000; Rajendrakumar et al., 2007).

Usually highly variable and widely dispersed, showing co-dominant inheritance and often flanked by unique sequences (Morgante et al., 2002).

SSR developed more recently through a different approach appear to be much more promising in cultivated groundnut genotypes (He et al., 2003; Ferguson et al., 2003).

Therefore it is suitable molecular markers for evaluating genetic diversity among cultivars and showed a high degree of polymorphism in many crop plants such as barley (Russell et al., 2000), pearl millet (Qi et al., 2001; Budak et al., 2003), sorghum (Smith et al., 2000; Casa et al., 2005), cotton (Chen et al., 2006), pear (Yamamoto et al., 2002) and peach (Arus et al., 2003). soybean (Song et al., 1999), wheat (Nagaoka and Ogihara, 1997), sweet potato (Huang and Sun, 2000) , maize, rice and tomatoes (Reif et al., 2006; Olsen et al., 2006; Caicedo et al., 2007), as well as in forage crops such as Perennial ryegrass (Jones et al., 2002a), white clover (Barrett et al., 2004), red clover (Sato et al., 2006).

The genome of red clover is extremely polymorphic due to its strongly self-incompatibile fertilization. The present varieties have developed mainly by mass selection, recurrent selection and natural selection (Taylor, 2008).

The use of breeding methods that improve specific traits while maintaining genetic diversity in a variety of red clover has resulted in abundant intra-population genetic diversity (Campos et al., 2001).

When various sets of cultivars or populations of red clover were analysed, large genetic diversity were determined among and within populations using morphological traits and molecular markers (Green et al., 2004; Dias et al., 2008).

This high level of genetic diversity in red clover is also evident in polymorphism analyses using RFLP, AFLP and microsatellite markers (Kolliker et al., 2003; Herrmann et al., 2005).

The development of simple sequence repeat (SSR) markers for red clover has allowed the analysis of these highly heterozygous varieties using highly informative co-dominant markers (Kolliker et al., 2006).

4. MATERIALS AND METHDOS

4.1. Experimental material and study site

The experimental material comprised of 40 red clover (*Trifolium pratense* L.) accessions from 17 different countries around the world, obtained from Institute of Field and Vegetable Crops, Novi Sad (Table 1).

The study was conducted on the research faild of the Institute of Field and Vegetable Crops, near Novi Sad,located at (latitude 45°33'N, longitude 19° 85'E), during the 2011. Research field-Rimski Šančevi is located at an altitude of 87 m above sea level.

4.2. Climate conditions

Novi Sad has a temperate continental climate, with four seasons. Autumn is longer than spring, with long sunny and warm periods. Winter is not so severe, with an average of 22 days of complete sub-zero temperature. January is the coldest month, with an average temperature of −1.9 °C (28.6 °F). Spring is usually short and rainy, while summer arrives abruptly. With an average annual, total rainfalls (1964-2011) are 614 mm, rainfall and mean monthly temperatures during the growing season (2011) (Table 2).

4.3. Soil conditions

Chemical analysis also showed that the soil was well-supplied with phosphorus and potassium, which is of particular importance for the production of red clover (Table 3).

Table1. List of 40 red clover accessions used in this study

Number	Accessions	0rigan	Status of accessions	Ploidy level
1	NCPGRU1	Ukraine	population	2n
2	NCPGRU6	Ukraine	population	2n
3	Keland-Bo	Bolivia	variety	2n
4	SA 2	Australia	population	2n
5	Kolubara	Serbia	variety	2n

6	Kohler	Switzerland	population	2n
7	Moser	Switzerland	population	2n
8	Changins	Switzerland	variety	2n
9	Bombi	Sweden	variety	2n
10	Fanny	Sweden	variety	4n
11	Pelly	Sweden	variety	4n
12	Atelo	Germany	variety	2n
13	Jubilatka	Germany	variety	4n
14	Matri	Germany	variety	4n
15	Nfg Mekra	Germany	variety	2n
16	Remy	Germany	variety	2n
17	Krano	Denmark	variety	2n
18	Crop	France	variety	2n
19	Pales	France	variety	2n
20	Skrzeszowicka	Poland	variety	2n
21	Viola,Hruszowska	Poland	variety	2n
22	Slovenska B	Czech Republic	variety	2n
23	Mir	Russia	variety	2n
24	Aberystwyth	UK	variety	2n
25	Rotonde, Kuhn	Holland	variety	2n
26	Teroba	Holland	variety	4n
27	Gkt Junior	Hungary	variety	2n
28	NS-Ravanica	Serbia	variety	2n

29	NCPGRU 8	Ukraine	population	2n
30	Valente	Italia	population	2n
31	Longevo	Italia	population	2n
32	Perseo	Italia	population	2n
33	Cremonese	Italia	population	2n
34	Brezova	Slovakia	population	2n
35	Rozbehi	Slovakia	population	2n
36	E-86	Bulgaria	population	2n
37	E-97	Bulgaria	population	2n
38	E-128	Bulgaria	population	2n
39	E-26	Bulgaria	population	2n
40	E-28	Bulgaria	population	2n

Table2. Rainfall and mean monthly temperatures during the growing season in 2011 (Source: Institute of Field and Vegetable Crops, Novi Sad, Laboratory for Agro Ecology).

Month	Rainfall (mm)	Temperature(^{o}C)
April	22.9	13.2
May	62.4	16.8
Jun	36.5	20.9
July	61.5	22.1
Total	183.3	73
Mean	45.8	18.2

Table3. Chemical properties of the soil (Source: Institute of Field and Vegetable Crops, Novi Sad, Laboratory for Agro Ecology).

Soil dept	CaCO3	PH		Humus	N	mg/100g soil	
(cm)	(%)	in H2O	in KCl	(%)	(%)	P2O5	K2O
30	2.52	8.25	7.28	2.58	0.19	32.4	30.6

4.4. Methods

In April 2011 the accessions were sown in a plot size of 21.6m x 73.8m, the experiment field is arranged as a complete randomized design(CRD) in three replicates, number of rows 28, length of row 7.2m , each red clover accession (genotype) was represented by a sample consisting of 30 plants (number of plant 10 plants per replication). The row-to-row spacing was 80x80 cm and the distances between blocks 1m. Hand weeding was also carried out in May 2011.

The morphological traits qualitative and quantitative observed was measured in mid-July 2011 for characterization and evaluation of the most important morphological-biological and agronomic traits in the field conditions table 3.2 for every accession five randomly selected individual plants were used for recording data using character descriptions adopted from UPOV criteria (UPOV, 1980).

Quantitative descriptors were taken as the mean value and then calculating the mean to get an overall figure per replicate, and subjected to multivariate hierarchical cluster analysis. The data analysis was done using the R software (R Core Team, 2012).

Fourty red clover accessions were grown under field conditions for study their morphological diversity (Table 1). Thirteen different quantitative and qualitative data (Table 4) were recorded, all the observations were recorded in five plants per accession per replication and the mean values were utilized for statistical analysis. The genetic diversity among the accessions was assessed using R software (R Core Team, 2012).

Table4. List of quantitative and qualitative characters recorded in the study

Character	Code	Description
Growth habit	GHA	visually assessed according to a five levels (1= erect, 3= semi erect, 5= medium,7= semi prostrate and 9= prostrate) scoring the plants before the plant cut.
Time of flowering	TFL	of each population was scored, as visually estimate in the field scoring
Yield of green mass (g) in growing	YGM	weight whole of plant without roots after cut from the ground per plant season
Yield of dry matter (g)	YDM	weight plant after air dry in growing season
Density of hairs in the stem	DOH	measured by touch during the fourth internodes from the Inflorescence
Intensity of white marks on the leaf	IWM	leaf mark was determined as present or absent.
Stem length (cm)	STL	measured from the base to the tip of the plant with the help of meter scale without stretching
Stem thickness	STD	at fourth internode diameter (mm) below terminal inflorescence
Number of internodes	NOI	Count of total internodes per plant start from top to down

Number of braches	NOB	the number of branches per plant were counted manually
Medium lamina length of medial leaflet (mm)	MLL	length of the first leaf under inflorescence
Medium lamina dimeter (mm)	MLD	width of the first leaf under Inflorescence
Leaf color	LCO	visually assessed

4.5. Statistical analysis of quantitative traits

In 2011 growing seasons using data of the 40 accessions the measurement of qualitative characteristics was done based on the scoring, eight morphological traits were subjected to.

Summary statistics per quantitative traits for descriptive statistical parameters such as mean, minimum, maximum value and standard deviation were calculated.

Mean comparison did by Student-Newman-Keuls (S-N-K) test (Table 6).

Analysis of variance (ANOVA) was carried out for the eight quantitative traits for the 2011 cropping seasons.

Phenotypic correlation coefficients were computed to examine the degree of association among the quantitative traits.

Multivariate analysis was employed using the appropriate procedure of the number cruncher statistical system (R Core Team, 2012).

Principal component analysis (PCA) of the traits was employed to examine the percentage contribution of each trait to total genetic variation and conducted to reduce the dimensionality of the data and enable visualization of the relationships among genotypes. Agglomerative hierarchical clustering was performed on the euclidean distance matrix utilizing the ward's linkage method these analyses were done using MEGA 4.0 software. PCA It can also handle variables of different types (nominal, ordinal and numerical) simultaneously and deal with relationships between variables.

It is based on the formula:

$Yij - yj = \lambda 1\ \text{Ɛ}i1\ \eta j1 + \lambda 2\ \text{Ɛ}i2\ \eta j2 + \text{Ɛ}ij$

Where Yij is the average yield of genotype i in environment j;

yj is the average yield over all genotypes in environment j; and λ 1Ɛi1ηj1 and λ 2Ɛi2ηj2 are collectively called the first principal component (PC1) and the second principal component (PC2). λ 1 and λ 2 are the singular values for the first and second principal components, PC1 and PC2, respectively; Ɛi1 and Ɛi2 are the PC1 and PC2 scores, respectively, for genotype i; ηj1 and ηj2 are the PC1 and PC2 scores, respectively, for environment j; and Ɛi2 is the residual of the model associated with the genotype *i* in environment *j*.

4.6. Cluster analysis

All the morphological observations were grouped by cluster analysis using the unweighted pair group method analysis (UPGMA) based on the similarity matrix of euclidean distances of the morphological data.To trace the relationship among the red clover varieties, the data were standardized before clustering and a dendrogram was constructed. The statistical analyses were performed using MEGA software (Tamura et al., 2007).
Euclidean or straight-line measure of distance was used for estimating genetic distance (GD) among accessions (Mohammadi and Prasanna, 2003). The matrix of average GD between two individuals i and j, having observations on phenotypic characters (p) denoted by x1, x2, , xp and y1, y2, , yp for i and j,respectively, was calculated using Euclidean distance, where:
GD (*i,j*) = [(x1-y1)2 + (x2-y2)2 + (xp-yp)2]1/2

4.7. Shannon Weaver diversity index for qualitative traits

Estimates of diversity indices for qualitative traits from different localities among red clover accessions were computed using the phenotypic frequencies **(H').**
The Shannon-Weaver diversity index to assess the phenotypic diversity for each character for all accessions. This index as described by Perry and Mclntosh (1991) is given as

$$SH = 1 - \sum_{i=1}^{n} pi \ log_e (pi).$$

Where *pi* is the frequency of the category *i* and *n* is the total number of categories. The SH penalizes redundancy at the category level and its maximum value (***log (n)***) is obtained when all classes are represented in equal proportions (*i.e. p1= p2=.....= p n* = 1/ n).

4.8. Molecular markers analysis

4.8.1. Simple Sequence Repeat (SSR) markers

Total genomic DNA was isolated according to the protocol of Rogers and Bendich (1988) from leaf tissue.

4.8.2. Solutions

An extraction buffer consisting of 2% hexadecyltrimethyl-ammonium bromide (CTAB) (w/v), 100 mM Tris (pH 8.0), 20 mM EDTA (Ph 8.0), 2 M NaCl, 2% polyvinylpyrrolidone (PVP - Mr 10,000, 5% β- mercaptoethanol (v/v), and 10 mM ammonium acetate was prepared. In addition, chloroform: octanol (24:1), 75% and 80% alcohol and a TE buffer consisting of 1 mM Tris (pH 8.0) and 1 mM EDTA (pH 8.0) were also needed.

4.8.3. DNA isolation and purification

Leaves were harvested and frozen immediately in liquid nitrogen.
They were used immediately, or frozen at –800 °C until required. A 4.0 g of leaf sample was ground in liquid nitrogen using a mortar and pestle pre-chilled to either –200 °C or –800 °C. Some liquid nitrogen was poured in just before adding the leaves. The pulverized leaves were quickly transferred to a liquid nitrogen prechilled, 50-mL falcon tube. 2% of pre-heated (650 °C) CTAB buffer (16 mL) containing 5% v/v β-mercaptoethanol and 2% PVP (Mr 10,000) was quickly added to the tube and stirred with a glass to mix. The tube was incubated at 650 °C for 5 min with frequent swirling. An equal volume of chloroform: octanol (24:1) was added and the sample centrifuged for not more than 5 s in a bench-top centrifuge (Biofuge 13, Heraeus) at room temperature to separate the phases.

The supernatant was carefully decanted and transferred to a new tube.The above steps, beginning with the addition of chloroform/octanol (24:1) and ending with decanting of supernatant, were repeated twice. The supernatant was precipitated with 2/3 volume of isopropanol. The precipitated nucleic acids were collected and washed twice with the buffer (75% ethanol, 10 mM ammonium acetate, TE). The pellets were air dried and resuspended in TE. The dissolved nucleic acids were brought to 2 m NaCl and re-precipitated using 2 volumes of 70% ethanol (If the pellet obtained was hard to re-suspend, this step was repeated one more time). The pellets were washed twice using 80% ethanol, dried and re-suspended in 100 μL of TE buffer. The tube was incubated at 650 °C for 5 min to dissolve genomic DNA, and RNase was then added.

4.8.4. Amount and purity of DNA

The yield of DNA per gram of leaf tissue extracted was measured using a UV-VIS Spectronic Genesys 5 (Milton Roy) spectrophotometer at 260 nm.The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. After quantification, the DNA was diluted to a concentration of 50ng/Nl for SSR diversity analysis. A total of 15 SSR primers were used for the analysis (Table 5).

4.8.5. PCR conditions and allele detection

PCR was performed in 10µl volumes containing approximately 25-50ng of template DNA, 1x PCR buffer (50mM KCl, 10mM Tris-HCl pH 8.3), 1.5mM MgCl2, and 0.15mM of each primer, 0.25mM dNTPs and 0.3U of Taq polymerase (Applied Biosystems). thermo cycling started with a denaturation step for 3 minutes at 94°C followed by 45 cycles of 1 minute at 94°C, 1 minute at 55°C, 2 minutes at 72°C and stopped after a final extension step of 72°C for 7 minutes. The fragment analysis was performed as a multi-loading assay analysing two markers simultaneously that was labelled by different ABI-dyes. Samples containing 0.5-1µl PCR products of each marker, 1µl internal size standard and 9µl Hi-Di formamide were separated using 36cm capillary arrays. Alleles were detected using the GeneScan/Genotyper software package of applied biosystems.

4.8.6. SSR primers

In all, fifteen primer pairs were used for genotyping (Table 5) The SSR markers used are distributed widely across the red clover linkage groups thus giving the comprehensive coverage of the red clover genome.

Table5. **Sequences of fifteen primer pairs of microsatellite markers tested in 40 accessions of red clover**

Primer	size	SSR motif	Forward primer (5′→3′)	Reverse primer (5′→3′)
RCS005	162	AC	CATTGTAGGTTATGTTTATCAGG	CCCAAAGCCTACAAGGAAAAG
RCS043	189	AAG	TCGCCACAAGGTCTCTTTTT	CGCTCTCTCTCTCTGCTTCA
RCS280	93	AAT	GAAGCAAAGCTGTGAAAGGG	GAGAATCTTGAGTGTGTGAAGGT
RCS008	178	AG	ATTCCCCCAATTTCCATCTC	TGCCCTGAAACCAAAAATGT
RCS084	156	AAG	CCTCATCATCAAATTCATTCTCA	AGCCAGAACCAGAACCTGAA
RCS167	238	AAC	CAGCAATCCAACGTTTCTGA	ATCATCACCAGCTTCAGCAC
RCS179	224	AAG	ATGGCTTCCTTCTTCACCCT	TCGACTGGGAAATCGATAGG
RCS278	202	AAC	GTCCATGAAGGCCGAAAATA	CAGAGGACCAGGAGGTGAAG
RCS125	200	ATC	TGCAAACTCCGCTTTATGC	CTCGCTGAAGGAGGAAACAG
RCS361	141	ATC	AAAGCACGTGAAGAAAATGGA	CCCTTCATCAATGGCTTTCT
RCS022	153	ATC	GGTAGTTTCTGACTTTCCCGTGT	TACAAAAGGGACCTGCTGCT
RCS001	135	AAAG	CCTCCTTGCATCATCTTTTC	AAAACTCGTTCGAGAGAGTG
RCS048	175	ATC	GAATGCCAAGACACCTGTGA	TCTCATCAAGGGAGGTGGTC
RCS065	178	GGT	TGTTGCTACAAGGCCAAAGA	AGCACTTTCGAACACAGCAA
RCS073	199	AAG	CGCAATCTTTCTTCTCATTTCA	TTCAACATGCAGGCTAAGAAAA

4.9. Statistical analysis

4.9.1. Data scoring

The PCR amplification using SSR markers was repeated twice. Consistent of banding pattern and polymorphism were used for scoring. The bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of a

matrix with ‘1’ and ‘0’, which indicate the presence and absence of bands in each accession respectively.

We carried out analysis of molecular variance (AMOVA) to partition the genetic variance between level of polyploidy, and status of cultivar.

Principal coordinate analysis (PCoA) of the traits was employed to examine the percentage contribution of each trait to total genetic variation, and performed to show the distribution of the genotypes in a scatter plot.

4.9.2. Cluster analysis

The scoring data in the form of binary values was used for the construction of dendrogram. The genetic associations between accessions were evaluated by calculating the Jaccard’s similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Jaccard, 1908). Similarity matrix was generated using the MEGA 4.0 software (using matrices from R software). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Unweighted Pair-Group method (UPGMA).

5. RESULTS AND DISCUSSION

Since only a wide genetic base gives the opportunity to select genotypes with a trait of interest, it is essential to understand the extent and distribution of genetic variation. Red clover is an outcrossing species with a high degree of gametophytic selfincompatibility (Taylor and Quesenberry, 1996) and populations are therefore composed of heterogenous individuals. Consequently, high levels of within-population variability are expected, decreased heterozygosity and heterogeneity of populations will decrease vigor and productivity (Tucak et al., 2008).

5.1. Genetic diversity analysis at morphological level

The present study was aimed to understand the genetic diversity and association mapping in red clover using morphological traits and simple sequence repeat (SSR) markers. 40 different red clover accessions representing diverse geographical location were studied for their genetic diversity at morphological and molecular level. The results of the experiments conducted are described.

Genotypes were also evaluated for morphological and quantitative traits including number of internodes, number of branch, stem length, stem thickness, middle of lamina length, middle of lamina width, yield of green mass and yield of dry matter per plants (Tables 6,7).

Morphological characterization is the first step in description and classification of genetic resources (Smith and Smith, 1989). Biodiversity among and within 48 red clover genotypes were investigated based on ten morpho-agronomic characters (flowering date, plant height, stem number, number of internode in the main stem, stem diameter, shape of medial leaflet, density of hairs in the main stem, width of medial leaflet, length of medial leaflet, (Asci, 2011)

Tables 6 . Mean dates are seprated by Student-Newman-Keuls (S-N-K) test for quantative traits for 40 red clover accessions

Genotype	NOI	NOB	STL	STD
NCPGRU1	5.18^{ebdacf†}	4.23^{ebdacf}	48.1^{ba}	5.12^{ba}
NCPGRU6	6.60^{a}	5.60^{a}	50.7^{a}	4.26^{ebdacf}
Kenland-Bo	5.42^{ebdacf}	4.38^{ebdacf}	50.6^{a}	4.57^{ebdacf}
SA 2	5.63^{ebdac}	4.65^{ebdacf}	45.6^{ba}	4.18^{ebdacf}

Kolubara	5.50^{ebdacf}	4.50^{ebdacf}	46.9^{ba}	3.11^{edf}
Kohler	5.04^{ebdacf}	4.04^{ebdacf}	43.5^{ba}	4.08^{ebdacf}
Moser	5.24^{ebdacf}	4.23^{ebdacf}	45.8^{ba}	3.35^{ebdcf}
Changins	5.07^{ebdacf}	4.07^{ebdacf}	44.0^{ba}	3.50^{ebdcf}
Bombi	5.26^{ebdacf}	4.26^{ebdacf}	45.3^{ba}	4.21^{ebdacf}
Fanny	6.28^{ba}	5.32^{ba}	48.8^{ba}	5.35^{a}
Pelly	5.30^{ebdacf}	4.33^{ebdacf}	45.7^{ba}	4.69^{ebdac}
Atelo	5.27^{ebdacf}	4.28^{ebdacf}	47.8^{ba}	4.43^{ebdacf}
Jubilatka	5.11^{ebdacf}	4.11^{ebdacf}	47.8^{ba}	4.99^{bac}
Matri	5.67^{ebdac}	4.63^{ebdacf}	46.8^{ba}	4.97^{bdac}
Nfg Mekra	5.92^{bdac}	4.92^{bdac}	46.6^{ba}	4.41^{ebdacf}
Remy	5.86^{bdac}	4.89^{bdac}	45.4^{ba}	3.94^{ebdacf}
Krano	5.81^{bdac}	4.87^{bdac}	44.8^{ba}	4.54^{ebdacf}
Crop	5.82^{bdac}	4.27^{ebdacf}	46.2^{ba}	3.99^{ebdacf}
Pales	5.20^{ebdacf}	4.18^{ebdacf}	47.2^{ba}	3.89^{ebdacf}
Skrzeszowick	6.08^{bac}	5.1^{bac}	49.2^{ba}	3.90^{ebdacf}
Hruszowska	5.58^{ebdacf}	4.57^{ebdacf}	45.6^{ba}	3.69^{ebdacf}
Slovenska B	5.18^{ebdacf}	4.17^{ebdacf}	43.7^{ba}	3.81^{ebdacf}
Mir	5.85^{bdac}	4.91^{bdac}	43.4^{ba}	4.37^{ebdacf}
Aberystwyth	5.51^{ebdacf}	4.51^{ebdacf}	41.9^{ba}	3.70^{ebdacf}
Rotonde-Kuhn	5.98^{bdac}	4.89^{bdac}	46.8^{ba}	4.69^{ebdac}
Teroba	5.19^{ebdacf}	4.22^{ebdacf}	39.7^{bdac}	4.42^{ebdacf}
Gkt Junior	5.51^{ebdacf}	4.51^{ebdacf}	44.4^{ba}	4.34^{ebdacf}
NS-Ravanica	4.81^{ebdcf}	3.82^{ebdcf}	42.2^{bac}	4.00^{ebdacf}
NCPGRU 8	5.39^{ebdacf}	4.42^{ebdacf}	45.3^{ba}	3.97^{ebdacf}
Valente	5.28^{ebdacf}	4.24^{ebdacf}	44.6^{ba}	4.06^{ebdacf}
Longevo	4.91^{ebdcf}	3.91^{ebdcf}	39.0^{bdac}	3.73^{ebdacf}
Perseo	5.30^{ebdacf}	4.27^{ebdacf}	46.2^{ba}	4.59^{ebdacf}
Cremonese	6.63^{a}	5.59^{a}	48.7^{ba}	4.19^{ebdacf}
Brezova	4.70^{ebdcf}	3.83^{ebdcf}	36.6^{bdec}	3.23^{edcf}
Rozbehy	4.40^{edf}	3.40^{edf}	32.2^{fdec}	3.19^{edcf}
E-86	4.13^{ef}	3.13^{ef}	30.6^{fde}	3.27^{ebdcf}

E-97	4.67 ^{ebdcf}	4.67^{ebdac}	31.7^{fdec}	3.52^{ebdacf}
E-128	4.50 ^{edcf}	3.67^{ebdcf}	26.5^{f}	2.78^{f}
E-26	4.00^{f}	3.00^{f}	28.0^{ef}	2.88^{ef}
E-28	4.47 ^{edcf}	3.57^{edcf}	38.6^{bdac}	3.63^{ebdacf}

*Means with the same letter are not significantly different.

NOI - number of internodes; **NOB-** number of braches; **STL-** stem length (cm) ; **STD-** stem thickness (mm); **MLL-** medium lamina length **(mm); MLD-** medium lamina diameter (mm); **YGM-** yield of green mass (g); **YDM-** yield of dry matter (g).

5.1.1. Number of internodes in red clover accessions

Considerable genetic variation was noticed for the average number of internodes ranged from 4.00 (E-26) to 6.63 (Cremonese) the total mean was 5.33 among genotypes, eighteen genotypes were found to possess greater number of internodes exceeding the total mean. Most of the accessions (22) were found to possess the number of internodes in the range of 4.00 to 5.30. (Table 6) appendix (1).

Analyzing 48 red clover populations, collected from 20 different locations in the Black Sea Region, Asci (2011) revealed that the number of internodes ranged from 5. 75 to 16. 25.

5.1.2. Number of branch in red clover accessions

The genetic variation for number of branches per plant ranged between 3.00 (E-26) and 5.59 (NCPGRU6). The total mean was found to be 4.35 and sixteen genotypes exceeded the general mean. The genetic variability of number of branches per plant is shown (Table 6) appendix (2).

5.1.3. Stem length in red clover accessions

Considerable genetic variation for plant height was exhibited by the red clover genotypes. The plant height of the genotypes ranged from 26.5cm (E-128) to 50.7cm (NCPGRU6) (Table 6) appendix (3).

Plant height (the same as the stem length) is a very important yield component. The average plant height for all analyzed ciutavars and population (Tucak et al., 2009) was 53.98 cm and varied from 32.65 to 66.69 Asci (2011) found wider range of variation for plant height (46.20 -92.0 cm) than in this study.

5.1.4. Stem thickness in red clover accessions

There was considerable genetic variation for number of Stem thickness per plant among the red clover accessions studied. The genotype E-128. recorded the lowest stem thickness per plant (2.78mm) and Fanny recorded the maximum number of stem thickness per plant of (5.35mm).Thirty six genotypes were found to possess an average of 3.00mm to 5.00mm stem thickness per plant (Table 6) appendix (4).

Concerning the main stem diameter, it changed like in this study between 2.18 mm and 4.19 mm (Asci, 2011).

Tables 7. Mean dates are seprated by Student-Newman-Keuls (S-N-K) test for quantative traits for 40 red clover accessions

Genotype	MLL	MLD	YGM	YDM
NCPGRU1	35.8^{a}	17.0^{bdac}	266.3^{ebdac}	66.3^{ebdac}
NCPGRU6	34.0^{ba}	14.5^{ebdghcf}	217.8^{ebdacf}	56.0^{ejbidhagcf}
Kenland-Bo	36.2^{a}	20.2^{a}	178.0^{ebdgcf}	64.9^{ebdacf}
SA 2	31.2^{bdac}	16.7^{ebdac}	189.6^{ebdacf}	57.3^{ebidhagcf}
Kolubara	31.9^{bdac}	13.2^{ebdghcf}	143.0^{edgf}	36.7^{ejidhgcf}
Kohler	32.6^{bac}	14.5^{ebdghcf}	119.5^{egf}	33.2^{ejidhgcf}
Moser	32.7^{bac}	14.2^{ebdghcf}	134.8^{edgf}	39.4^{ejidhgcf}
Changins	32.7^{bac}	14.2^{ebdghcf}	133.7^{edgf}	37.4^{ejidhgcf}
Bombi	33.3^{bac}	15.3^{ebdghcf}	301.3^{ba}	87.1^{a}
Fanny	34.4^{ba}	18.0^{ba}	337.0^{a}	83.1^{ba}
Pelly	32.0^{bac}	16.0^{ebdgcf}	232.0^{ba}	58.8^{ebdhagcf}
Atelo	33.0^{bac}	14.9^{ebdghcf}	137.8^{edgf}	38.2^{ejidhgcf}
Jubilatka	33.4^{bac}	17.3^{bac}	138.5^{edgf}	37.4^{ejidhgcf}
Matri	33.0^{bac}	16.6^{ebdacf}	173.5^{ebdacf}	45.3^{ejidhgcf}
Nfg Mekra	32.0^{bac}	13.3^{ebdghcf}	157.6^{edgcf}	46.7^{ejidhgcf}
Remy	31.5^{bdac}	13.7^{ebdghcf}	142.8^{edgf}	46.4^{ejidhgcf}
Krano	28.1^{ebdac}	13.4^{ebdghcf}	276.3^{bdac}	74.0^{bac}
Crop	32.7^{bac}	13.7^{ebdghcf}	124.3^{edgf}	38.5^{ejidhgcf}
Pales	33.8^{ba}	14.2^{ebdghcf}	139.4^{edgf}	38.0^{ejidhgcf}

Skrzeszowick	32.4^{bac}	13.2^{ebdghcf}	193.4^{ebdacf}	56.3^{ejbidhagcf}
Hruszowska	32.6^{bac}	14.3^{ebdghcf}	189.1^{ebdacf}	51.2^{ejbidhgcf}
Slovenska B	32.7^{bac}	13.3^{ebdghcf}	125.2^{edgf}	37.0^{ejidhgcf}
Mir	28.8^{ebdac}	14.3^{ebdghcf}	289.9^{bac}	68.5^{bdac}
Aberystwyth	27.9^{ebdac}	12.0^{edghf}	150.8^{edgcf}	45.7^{ejidhgcf}
Rotonde-Kuhn	32.4^{bac}	15.0^{ebdghcf}	177.8^{ebdacf}	46.7^{ejidhgcf}
Teroba	28.5^{ebdac}	15.6^{ebdghcf}	154.3^{edgcf}	39.7^{ejidhgcf}
Gkt Junior	31.5^{bdac}	13.5^{ebdghcf}	172.5^{ebdacf}	49.3^{ejbidhgcf}
NS-Ravanica	31.9^{bdac}	14.4^{ebdghcf}	118.8^{egf}	29.5^{ejidhgf}
NCPGRU 8	32.7^{bac}	13.9^{ebdghcf}	124.7^{edgf}	28.1^{ejihgf}
Valente	31.6^{bdac}	14.3^{ebdghcf}	138.6^{edgf}	38.0^{ejidhgcf}
Longevo	29.1^{ebdac}	13.4^{ebdghcf}	79.0^{gf}	24.5^{jihg}
Perseo	31.4^{bdac}	15.5^{ebdghcf}	192.9^{ebdacf}	44.2^{ejidhgcf}
Cremonese	28.6^{ebdac}	13.1^{ebdghcf}	179.4^{ebdecf}	61.0^{ebdagcf}
Brezova	25.3^{ebdc}	10.8^{h}	48.3^{g}	22.8^{jihg}
Rozbehy	23.6^{ed}	11.8^{eghf}	39.7^{g}	18.0^{ji}
E-86	25.3^{ebdc}	12.3^{edghcf}	75.6^{gf}	25.6^{jihgf}
E-97	23.2^{e}	10.5^{h}	80.8^{gf}	19.8^{jih}
E-128	24.5^{edc}	11.3^{gh}	53.3^{g}	16.4^{j}
E-26	23.7^{ed}	11.4^{ghf}	80.0^{gf}	19.9^{jih}
E-28	29.8^{ebdac}	15.1^{ebghcf}	109.5^{gf}	29.9^{ejidhgf}

5.1.5. Medium lamina length in red clover accessions

Considerable genetic variation was recorded among the red clover genotypes for medium lamina length genotypes ranged between 23.16mm (E-97) and 36.23mm (Kenland-Bo). The total mean medium lamina length was 30.80mm and twenty seven genotypes exceeded in the mean value (Table 7) appendix (5). Medium leaflet length ranged from 13.3 to 44.3 mm (Asci, 2011).

5.1.6. Medium lamina diameter in red clover accessions

The red clover genotypes exhibited a higher degree of variability for medium lamina diameter the genotype E-97 recorded the lowest medium lamina diameter of 10.50mm and the genotype Kenland-Bo was found to possess the medium lamina diameter of 20.23mm.

The general mean of medium lamina diametee among the genotypes was 14.25mm and about 36 genotypes were found to possess the medium lamina diameter of 12 mm to 18 mm (Table 7) appendix (6).

Asci (2011) revealed that average medium leaflet width of 48 red clover genotypes was measured as 15.7 mm. It is similar to value achieved in this study.

5.1.7. Yield of green mass in red clover accessions

The red clover accessions exhibited a high level of genetic variation for the trait yield green mass. The genotype Rozbehy recorded the lowest yield of green mass 39.73g and the genotype Fanny recorded the highest yield of green mass 337g. The general mean of the genotypes was 157.92g and fifteen genotypes exceeded the general mean (Table 7) appendix (7).

(Tucak et al., 2009) analyzed 30 red clover cultivars and populations from 11 countries, including two populations from Croatia and found a high level of variatiom fpr the yield green mass. The highest average yields of green mass and dry matter were obtained for the Croation breeding population Pop 11 (1008.6 and 262.73 g plant) and were 52% higher compared with average yields for all materials.

5.1.8. Yield of dry matter in red clover accessions

Yield of dry matter among the red clover accessions ranged between 16.4g and 87.10. The general mean of the genotypes was 43.93g and nineteen genotypes exceeded the general mean (Table 7) appendix (8).

The big difference between the minimum and the maximum yield of dry matter (30.58 and 316.01 g. respectivily), obtained in the study Asci (2011) indicate that large variation existed among the red clover genotypes.

In general, the studied red clover accessions showed significant variation in phenotypic characters, indicating that the accessions had high genetic diversity which should allow development of new genotypes of desired traits through selection and crossing programmers.

5.2. Descriptive statistics of the morphological data

Substantial variability among forty genotypes was also revealed by big difference between minimum and maximum values as well as high standard deviations for eight quantitative characters are given in (Table 8). The standard deviation ranged from 0.60

(number of internodes) to 68.82 (yield of green mass). The number of internodes ranged from 4.00 (E-26) to 6.63 in (NCPGRU6). Number of braches among the genotypes ranged from 3.00 (E-26) to 5.6 (NCPGRU6). The minimum (26.50 cm) and maximum (50.7 cm) stem length was observed in E-128 and NCPGRU6 respectively. The stem thickness among genotypes varied from 2.78 (E-128) to 5.35 (Fanny).

Medium lamina length, medium lamina diameter showed a considerable of variation. Medium lamina length was minimum (23.15 mm) in E-97 and maximum (36.24 mm) in Kenland-Bo.

Medium lamina diameter was (10.52mm) and (20.21mm) in E-27 and Kenland-Bo respectively. The big difference between the minimum and the maximum yield of green mass weight (39.73 g and 337 g) in Rozbehy and genotype Fanny respectively indicate that large variation existed among the genotypes . Furthermore, the yield of dry matter also showed considerable variation among genotypes ranged from (16.4 g) in E-128 to (87.1g). In Bombi the standard deviation shows the amount of variation for each trait among the accessions. A higher variation for a character in the breeding materials correlates with a greater ability for its improvement through selection.

Table8. Means, standred deviation, maximum and minimum values for 8 quantitative trait for 40 red clover accessions

Trait	Mean	Std.dev.	Minimum	Maximum
NOI	5.33	0.60	4.00	6.63
NOB	4.35	0.58	3.00	5.60
STL	43.31	6.04	26.50	50.70
STD	4.04	0.61	2.78	5.35
MLL	30.80	3.36	23.15	36.24
MLD	14.25	1.98	0.52	20.21
YGM	157.92	68.82	39.73	337.00
YDM	43.93	17.41	16.40	87.10

5.3. Analysis of variance for quatitative traits

Wide variation among genotypes for all the traits evaluated was detected. The mean compression for the accessions as shown in table 8. Analysis of variance showed significant differences among genotypes for all eight quantitative characters, indicating the presence of substantial variability among tested genotypes table 9.

Table9. Analysis of variance (ANOVA) for 8 quatitative traits for 40 red clover accssions

Trai	df	SS	MS	*F*
NOI	39	42.1	1.08	4.12**
NOB	39	38.8	0.99	3.65**
STL	39	4272	110	6.26**
STD	39	43.5	1.11	3.38
MLL	39	1325	34.0	4.24
MLD	39	459.3	11.8	4.56
YGM	39	554185	14210	5.79
YDM	39	35476	910	5.70

5.4. Correlation analysis

In order to obtain the level of relationship between studied traits, we calculated of correlation among red clover quantitative traits by non-parametric Spearman correlation coefficient.

According to the correlations values of internodes had the strongest genetic correlation with number of branch (r = 0.95**, Table 10). Appendix (9) this correlation was high very significant, strong relationship also was found between number of internodes, on one side, and stem length (r = 0.80**), yield of dry matter (r = 0.69**), yield of green mass (r = 0.65**), on the other side. Medium, positive correlations were found between, number of internodes and medium lamina length (r = 0.54**), and medium lamina diameter (r = 0.34*).

Regarding the correlations, the highest value of phenotypic coefficient of correlation, was determined between number of branch and stem length (r = 0.70**, Table 10). High significant, strong relationship was found between number of branch and other studied traits yield of green mass (r = 0.64**) and yield of dry matter (r = 0.66**), medium lamina length, where we obtained medium correlation (r = 0.42**), and stem thickness diameter(r = 0.54**). Medium lamina diameter was in lowest correlation and non significant with number of branch ($r = 0.25^{ns}$).

Between other studied traits, the strongest correlations, which showed high significant, was found between stem length and medium lamina length (r = 0.89**, Table 10). High significant, strong relationship also was found between stem length, on one side, and stem thickness diameter (r = 0.70**), medium lamina diameter (r = 0.66**), yield of dry matter (r = 0.67**) and yield of green mass (r = 0.64**).

A number of researchers (Wioncek et al., 1976, Vasiljevic et al., 2006) have found significant correlations betwen green forage yield and stem length in red clover, which suggests that selection for a longer stem may lead to increased green mass yields.

Correlations between stem thickness diameter, on one side, and medium lamina diameter was strong correlations (r = 0.77**, Table 10). High significant, strong relationship, also was found between stem thickness diameter, and yield of green mass (r = 0.72**), yield of dry matter (r = 0.67**), yield of green mass (r = 0.64**).

The greatest value of phenotypic coefficient of correlation was obtained between medium lamina length and medium lamina diameter (r = 0.76**, Table 10).

On the other side, medium, positive correlations were found between medium lamina length, and yield of green mass(r = 0.54**), yield of dry matter(r = 0.54**).

According to the analysis, the medium correlations was found between medium lamina diameter and yield of green mass (r = 0.58**, Table 10) and yield of dry matter (r = 0.58**).

High significant, strong relationship also was found between yield of green mass, on one side, and yield of dry matter (r = 0.96** Table 10).

Regarding the correlations (Asci, 2011) positive relationship existed between stem length and yield of dry matter (0.870**) and between stem length and number of internodes (0.296**).

Table10. Associations among red clover quantitative traits by non-parametric Spearman correlation coefficient

	NOI	NOB	STL	STD	MLL	MLD	YGM
NOB	0.95**						
STL	0.8**	0.7**					
STD	0.57**	0.54**	0.7**				
MLL	0.54**	0.42**	0.89**	0.63**			
MLD	0.34*	0.25^{ns}	0.66**	0.77**	0.76**		
YGM	0.65**	0.64**	0.64**	0.72**	0.54**	0.58**	
YDM	0.69**	0.66**	0.67**	0.68**	0.54**	0.58**	0.96**

5.4. Principal component analysis (PCA)

Principal component analysis (PCA) of the quantitative data was performed to investigate the importance of different traits in explaining multivariate polymorphism and the most commonly is useful tools for screening accessions , and thus guide in the choice of parents for hybridization (Chozin, 2007).

Biplot was used to assess the GGE biplot method (Yan et al., 2000) to assess the patterns of relations among morphological attributes, genotypes and their interactions and conducted in the dimension of first two principal components (PC1 and PC2), using a singular-value decomposition procedure.

Applied the GGE biplot model accounted for 83.7% of the total variation of the standardized data, consisting of 69.2 and 14.5 % of variance attributable to PC1 and PC2 respectively, suggesting that meaningful deductions can be made from the data using the GGE biplot methodology (Yan et al., 2000). The accessions were separated based on principal component analysis (PCA), the most commonly applied, and cluster analysis, are

useful tools for screening accessions (Talhouk et al., 2000; Cordeiro et al., 2001;Thakur et al., 2005; Chalak et al., 2007; Sorkheh et al., 2009, 2010).

According to quantitative traits the variation studied through principal component analysis revealed that. The traits, which contributed more positively to PC1, were number of internodes, number of branch, yield of green mass and yield of dry matter.

PC2 was contributed more positively by stem length, stem thickness, medium lamina length, and medium lamina width.

Based on the states of accessions the majority of populations grouped to gather and this is agree with UPGMA tree and this populations originated from Bulgaria and Slovakia, and characterized by lowest number of internodes and branches, shortest stem length and stem thickness, narrowest medium lamina length and lamina diameter, lowest green mass and dry matter yield. But other cultivars clustered around the center of the graph (figure 3, A).

According to ploidy level the accessions 2n (diploid) scattered in all the quarterlies which showed the high level of genetic diversity. While 4n (tetraploid) distributed around the center of the graph (figure 3, B).

In the principal components analyses of 22 red clover accessions, performed on seven statistically-significant morphophysiological traits, the first (PC 1) and the second (PC 2) axes accounted for 57 and 22 %, respectivily of the total variation (Pagnotta et al., 2011).

Trait eigenvectors indicated that PC 1 was mainly a positive indicator of erect habit and of characteristics contributing to high medium-term forage yield and high seed yield (tall and nimerous stems, large leaves, many flowers per inflorescence, reflecting the significant correlations found among these traits. PC 2 was mainly a positive indicator of late flowering and of high frequency of plants with leaf marks (Pagnotta et al., 2011).

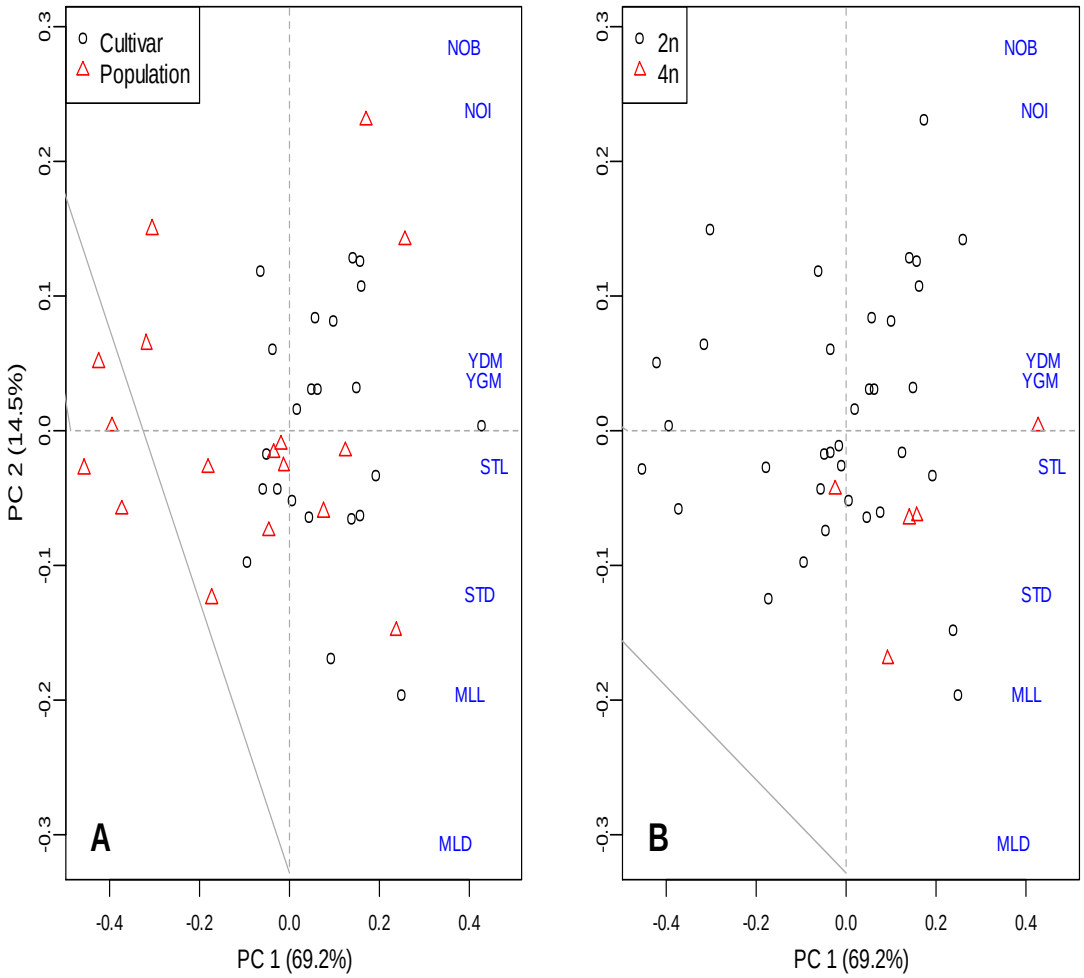

Figure 3 A, B. Biplot of first and second principal component.
A. states of accessions **B.** ploidy level of accessions.

Based on full accessions name there are wide phenotypic diversity among red clover accessions studied was further explained by the PCA biplot (figure 4). The PCA biplots provide an overview of the similarities and differences between the quantitative traits of the different accessions and of the interrelationships between the measured variables. The biplot demarcated the accessions with characteristics explained by the first two dimensions. The PCA grouped the accessions into groups over the four quadrants based on the quantitative traits. The accessions remained scattered in all four quadrants, showing large genetic variability for the traits studied. Accessions which overlapped in the principal component axes had similar relationships in the traits.

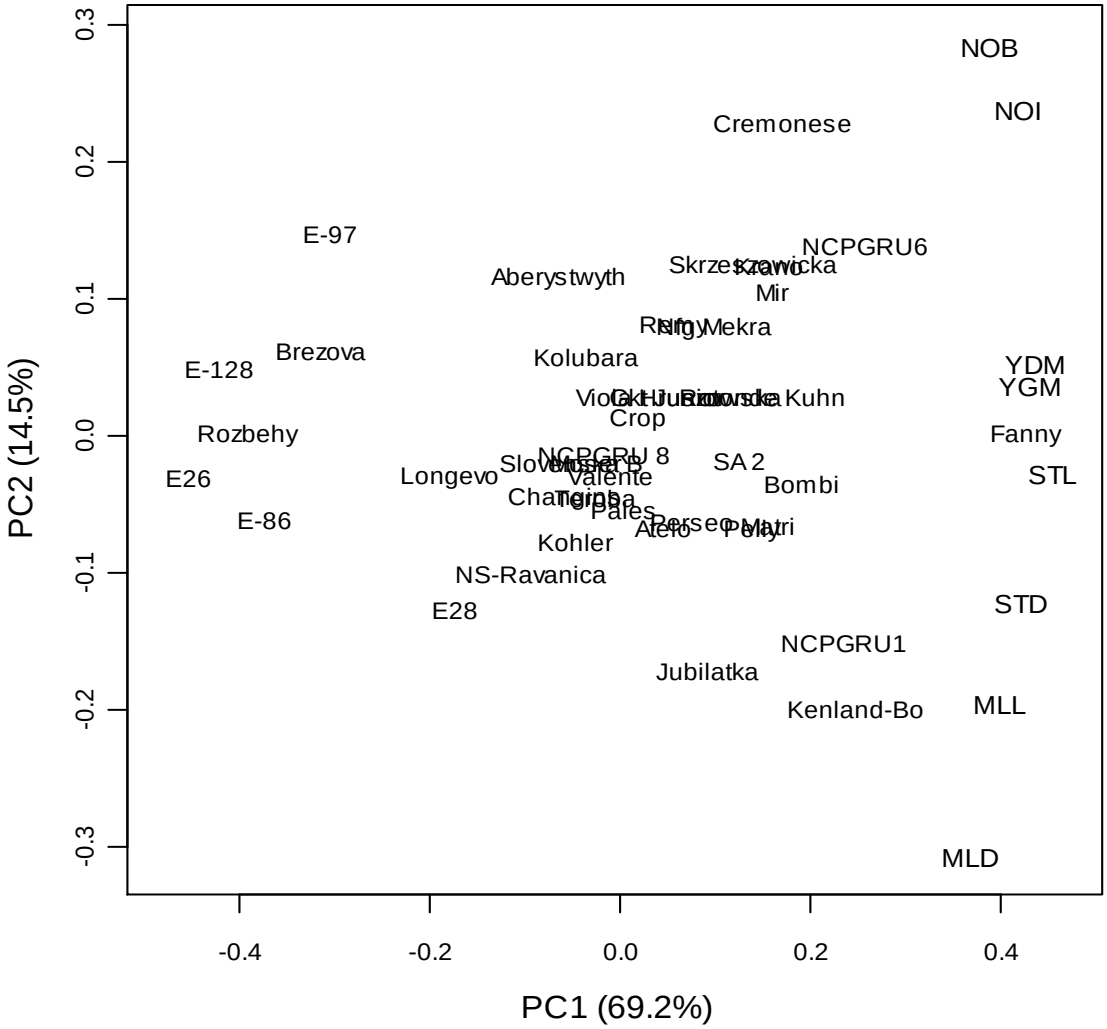

Figure 4. Principal component score plot of PC1 and PC2 describing the overall variation among red clover accessions estimated using phenotypic character data.

According the origin grouping the first two principal components revealed that the accessions were scattered in all the quarters, which showed the high level of genetic diversity in the evaluated genotypes. Among the16 counters the genotypes ITA (Italy), UKR (Ukraine) ,BGR (Bulgaria), SVK (Slovakia), SRB (Serbia), DEU (Germany), UKR (Ukraine), and BOL (Bolivia) was found diversely scattered in the scatter plot (Figure 4). Ordination and classification of the 22 red clover accessions (Pagnotta et al., 2011) based on morphopysylogical traits clearly followed the type of germplasm and its geographic origin.

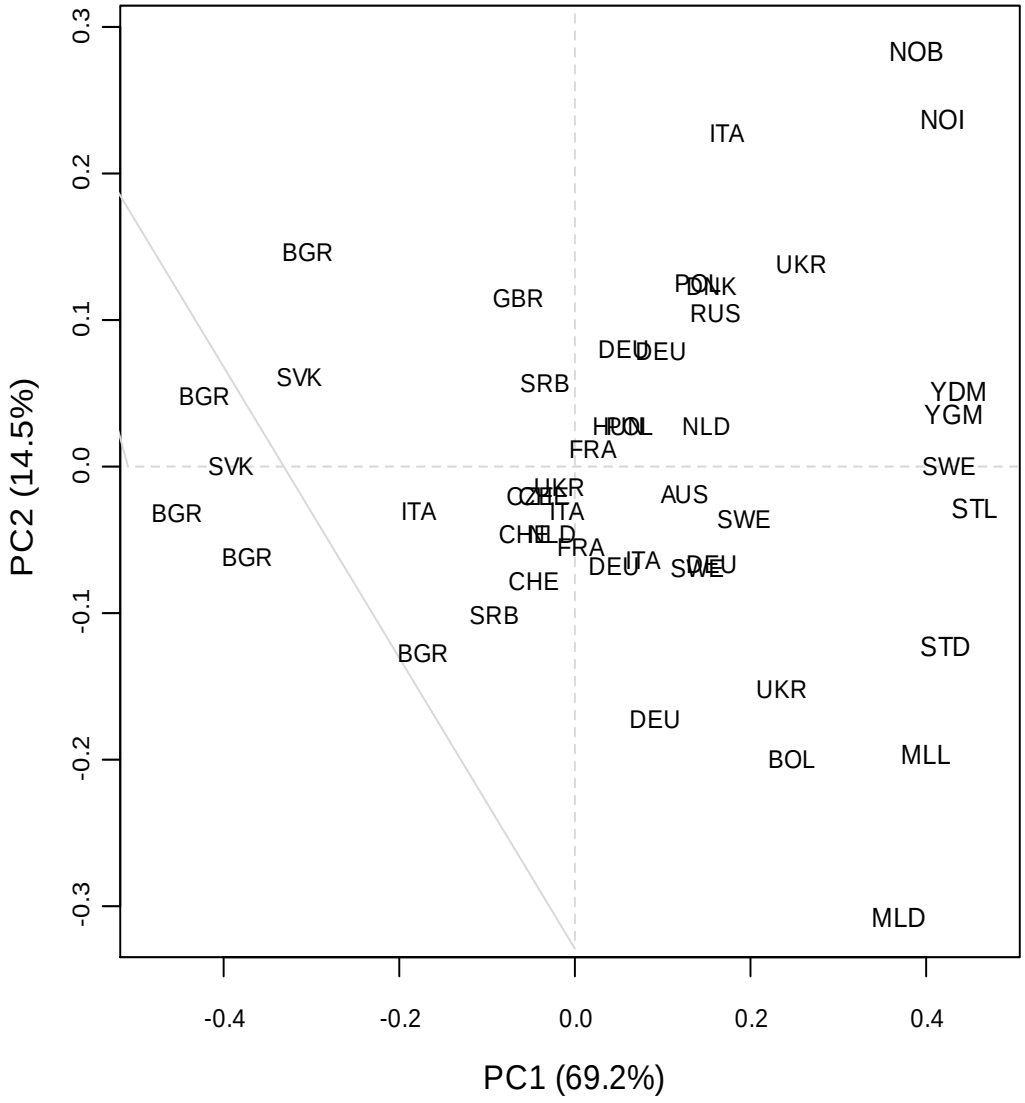

Figure 5. Biplot of first and second principal component (origin grouping). Abbreviations as given in table 1.

5.5. Cluster analysis

5.5.1. Genetic distance and cluster analysis

In order to determine the similarities or differences among genotypes, a cluster analysis was performed as well. The statistics of the cluster analysis, based on the eight morpho-agronomic traits, allowed the identification of six basic groups (figure 6). According to the cluster analysis, genotypes Moser and Changing seemed to be close to each others. Cluster IV contained the largest number of genotypes collected from nine different locations. It was noticed that there was statistically significant differences among and within the gonotypes concerning locations. In general, the genoytpes collected from near regions,

were clustered in the same groups. Furthermore estimates of genetic distances matrix based on phenotypic characters for all pair-wise combinations of (40 × 39)/2 = 780 for the 40 red clover accessions. Genetic distances from 10.35 to 0.56 were observed in the pair-wise combinations, indicating that the accessions were diverse for the phenotypic characters measured. The minimum genetic distance of 0.56 was recorded between accessions Changings and Moser. On the other hand, the highest genetic distance of 10.35 was recorded between accession E26 (Bulgaria) and Fanny accession (Sweden), indicating that there was a high genetic diversity between the accessions.

Cluster (segmentation) analysis for phenotypic traits shows a clear demarcation between red clover accessions (figure 6). Furthermore differences among clusters by summering cluster means for the eight quantitative traits. Based on these traits, the accessions were grouped into different clusters. The dendrogram split the accessions into distinct five main clusters and a singleton. Yet, it can be maintained that plants of the tetraploid varieties, Pelly, Matri, and Teroba split into two sub-clusters. The first main cluster was produced at a genetic distance of 2.36 and included the accessions NCPGRU 1, Bombi, Kenland-Bo and Jubilatka.

Cluster I characterised by the widest medium lamina length ,medium lamina diameter and the longest stem length, medium number of internodes and branch, high stem length and thickness , high green mass and dry matter yield.

The II main cluster was also formed at a genetic distance of 3.5 and comprised of four accessions NCPGRU6, Cremonese, Krano, and Mir these clusters characterized by biggest number of internodes, and number of branches, medium stem length and thickness, medium medium lamina length and diameter , high green mass and dry matter yield.

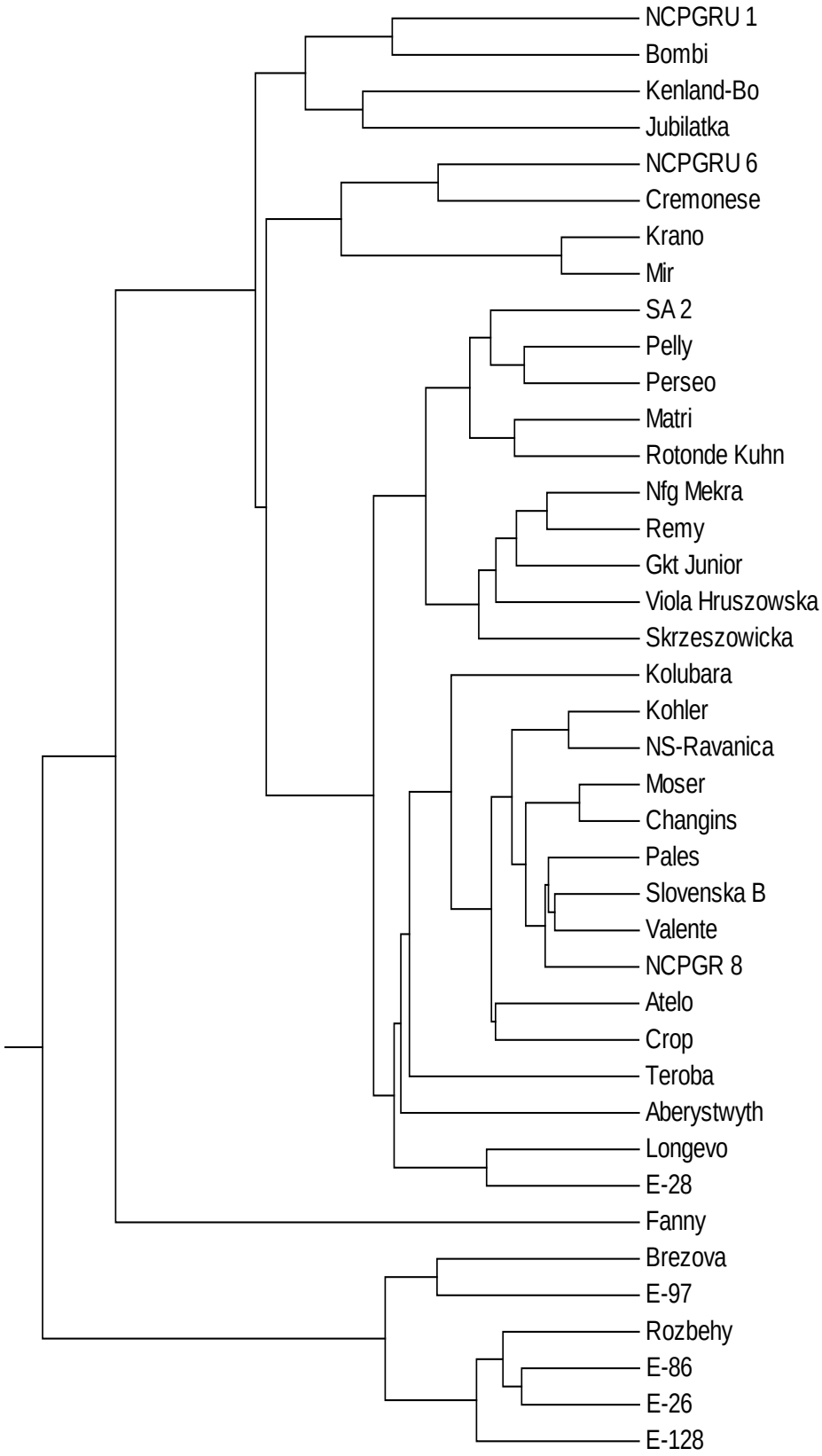

Figure 6. Dendrogram of 40 red clover accessions revealed by UPGMA cluster analysis based on phenotypic data.

The III cluster

consisted of ten accessions SA 2, Pelly, Perseo, Matri, Rotonde Kuhn, Nfg Mekra, Remy, Gkt Junior, Viola Hruszowska and Skrzeszowicka this cluster grouped the accessions with medium number of internodes, medium number of branch , high stem length and thickness , medium medium lamina length and diameter , medium green mass and dry matter yield.

Cluster IV comprised of fifteen accessions, Kolubara, Kohler, NS-Ravanica , Moser, Changins, E-28, Atelo, Crop, Pales, Slovenska B, Aberystwyth,
Teroba, NCPGRU8, Valente, and Longevo characterized by medium number of internodes and number of branches, medium of stem length, low stem thickness, low medium lamina length low yield of green mass and yield of dry matter.

Cluster V contained only one accession, Fanny and formed at a genetic distance of about 4.67 and was from Sweden. This cluster consisted of an accession with the highest number of internodes, biggest number of branch, longest stem length, thickest stem thickness, highest medium lamina length, widest medium lamina diameter, and biggest green mass and dry matter yield. Accession Fanny was not included in any of the clusters and grouped as a singleton and Stood individually as a separate cluster and this indicates that it was phenotypically dissimilar from the other accessions. Accession Fanny revealed the highest genetic dissimilarity coefficient value of 4.6 and appeared as the most divergent accession. This indicated that the accessions included in this study could be valuable sources of genetic variability in the red clover improvement programs.

Cluster VI consisted of six accessions E-86, E-97, E-128, and E-26 which originated from Bulgaria and two accessions Brezova and Rozbehy which originated from Slovakia. This cluster grouped the accessions with lowest number of internodes and branches, shortest stem length and stem thickness, narrowest medium lamina length and lamina diameter, lowest green mass and dry matter yield.

The statistics of the cluster analyses, based on the ten morpho-agronomic traits, allowed the identification of eight basic groups (Asci, 2011). In general, the genotypes collected from near regions wege clustered in the same groups.

Tucak et al. (2009) analyzed 30 red clover cultivars and populations using seven morpho-agronomic traits and identified six clusters. Roso and Pagano (2005) evaluated 39

introduced and naturalised populations and detected winter forage yield and seed production as the most important traits to explain variation between populations.

Table11. The summary of cluster means of eight quantitative traits for the red clover accessions based on data set

	Cluster means						
Characters	I	II	III	IV	V	VI	means
Number of Internodes	5.24	6.22	5.68	5.19	6.28	4.4	5.48
Number of Branches	4.22	5.24	4.17	4.92	5.32	3.6	4.57
Stem Length	47.95	46.9	46.20	43.76	48.8	30.93	44.16
Stem Thickness	4.72	4.34	4.34	3.84	5.35	3.14	4.28
Medium Lamina Length	34.67	29.87	32.01	31.57	34.4	24.26	31.14
Medium Lamina diameter	17.3	13.82	15.76	12.2	18.0	11.35	14.73
Yield of Green mass	221.02	240.85	182.12	128.89	337	62.59	195.41
Yield of Dry matter	63.92	64.87	50.3	35.58	83.1	20.41	53.03

5.6. Diversity index for qualitative traits

Genetic diversity among 22 red clover accessions was investigated based on some qualitative traits (vegetative growth habit, flowering gate, frequency of plants without leaf marks, flower colour (Pagnotta et al., 2011).

Estimates of phenotypic diversity has described by Shannon Weaver diversity index as described by (Perry and Mclnntosh, 1991). This criterion is suitable for evaluating core collections using categorical data; it has been used extensively in the literature.

For here qualitative traits over all 40 red clover accessions are shown in (Table 12). Individual traits showed a different pattern of variation among accessions, estimates of

phenotypic diversity indices (H') for individual traits varied from 3.595 for time of flowering to 3.673 for leaf color with an overall mean phenotypic average diversity index was 3.631.
The diversity values for the characters showed narrow variability among them. Thus, the diversity among accessions varied depending on the characters. According to (Brown and Weir, 1983) this index is used in genetic resource studies as a convenient measure of both allelic richness and allelic evenness when using genetic data.
Significant ($P<0.05$) genetic variation among 22 accessions (16 Italian natural populations, 4 traditional commercial ecotypes, 2 varaties) was found for all morphophysiological traits involved some qualitative characters: vegetative growth habit, flowering date, frequency of plants without leaf marks, except flower colour (Pagnotta et al., 2011). Commercial ecotypes, variaties and a few natural populations which originated in medium-to-low altitude, productive grasslands of northern Italy tended towards erect growth habit. Prostrate habit was was displayed by several natural populations from Sarinia`s pastures, which were characterised by the presence of leaf mark. Late flowering was a feature of all natural populations collected from pastures (Pagnotta et al., 2011)

Table12. Estimates of diversity indices for qualitative traits from different localities among red clover

Trait	**Abbreviation**	**Trait states**	***Hj***
Time of flowering	TFL	1 - 3 - 5	3.595
Growth habit	GHA	5 - 3 - 7 - 9	3.603
Density of hairs	DOH	1 - 3 - 5	3.652
Leaf color	LCO	3 - 5 - 7	3.673
Intensity of white Marks	IWM	1 - 3 - 5	3.636
Average diversity index			3.631

Hj – Shanon diversity index

5.7. Cluster analysis based on UPOV data

The International Union for the Protection of New Varieties of Plants (UPOV) is an organization that protects breeder's rights publishing a guide with morphological descriptors to conduct a new variety's registration test. In this study UPOV data for morphological descriptors, across the red clover accessions studied the genetic similarity index was calculated based on Rogers and Tanimoto's coefficient. The genetic distance indices ranged from 0.00 to 1.00.

Cluster analysis of similarity estimates was performed to construct a UPGMA tree showing overall genetic relationship among red clover accessions (figure 7). The UPGMA dendrogram constructed based on Rogers-Tanimoto distance revealed that red clover accessions separate into five clusters, at a mean distance of 0.582. Cluster 5, included accessions Brezova, Rozbehy, and E-28 , cluster 4, included accessions SA 2 ,Fanny and Jubilatka , cluster 3, comprise only one accession E-26 , cluster 2, It can be divided into two sub clusters: sub cluster 1, which has close relationship between accessions Kenland-Bo, Kohler, Changins, Atelo, Remy, Crop, Slovenska B, Mir, Rotonde Kuhn, Teroba, Viola Hruszowska, Gkt Junior, Perseo, E-86 and E-97 and sub cluster 2, with twelve accessions NCPGRU 6, NCPGRU 8, Moser, Nfg Mekra, Pales, Longevo, Aberystwyth, Cremonese, Kolubara, Valente, Krano and Skrzeszowicka.

Cluster 1, included NCPGRU 1, Bombi, NS-Ravanica, E-128, Pelly and Matri

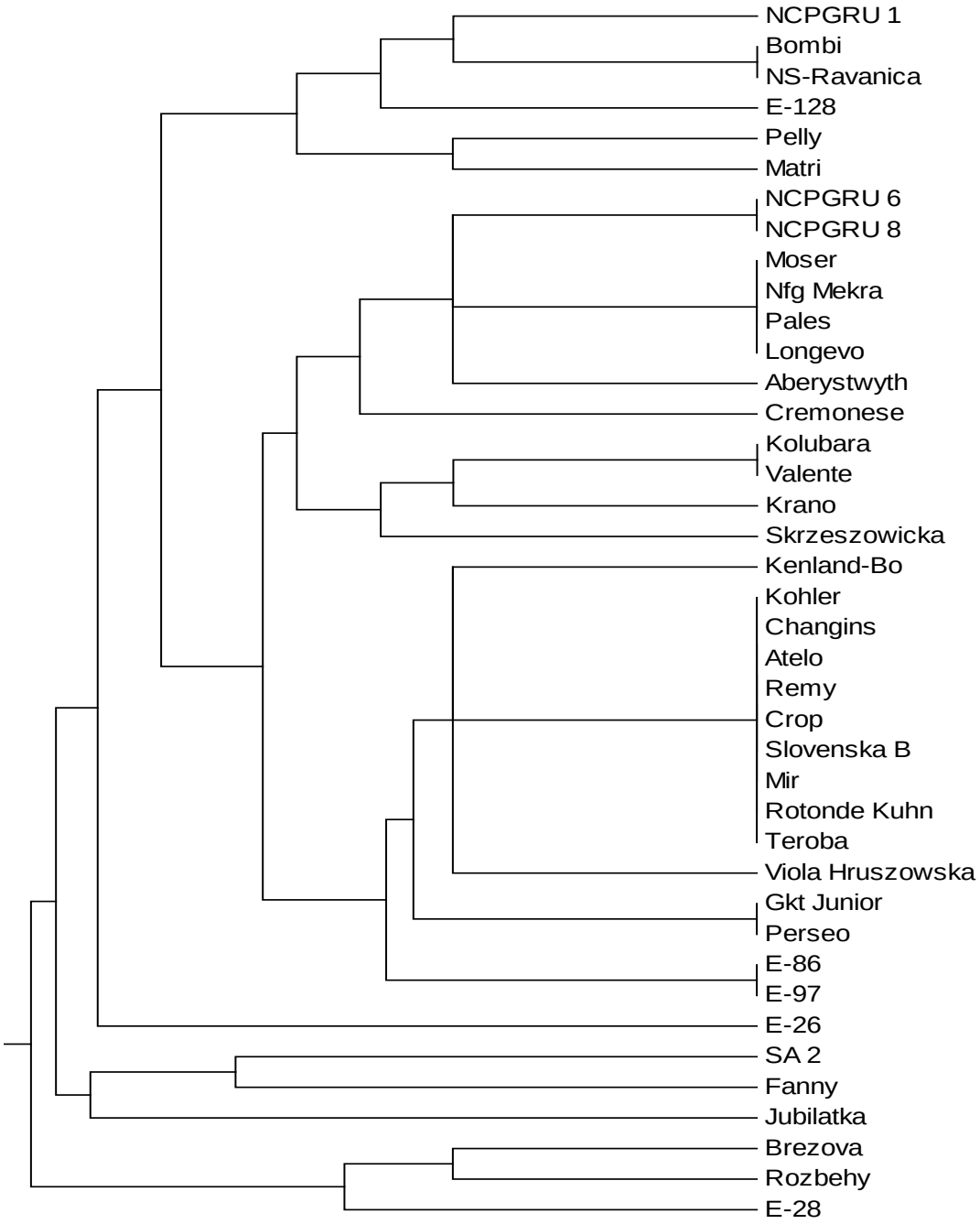

Figure 7. Dendrogram of 40 red clover accessions revealed by UPGMA cluster analysis based on UPOV data.

Flowering date, plant height, stem number, number of node in the main stem , maim stem diameter, shape of medial leaflet and density of hairs in the main stem were determined according to UPOV, 2001 criteria (Asci, 2011). The statistics of the cluster analyses based on these traits, allowed the identification of eight basic groups. Greene et al., (2004) studied 33 wild red clover populations' using15 morphological traits and found that flowering time and winter survival contributed to group population into three classes. Rosso and Pagano

(2005) analyzed cultivars and naturalized populations using 14 morphological traits and sugested that winter forage yield, flowering date and seed yield as the most important traits to explain variation between these populations. Dias et al., (2008) analyzed 58 populations using 21 morphological traits and found relation between high intensity of blooming and low persistence. All the populations were separated into five distinct clusters of germplasm, mainly characterised by blooming date, persistency, and dry matter production and growth habit.

5.8. Mantel test

The simple Mantel test only allows the use of two dissimilarity matrices. Many methods have been proposed for the inclusion of additional matrices. The most commonly used is the partial correlation approach, so that the Mantel statistic is the partial correlation of A and B given all other explanatory variables. The partial Mantel test has frequently been used to examine the relationship between vegetation composition and environmental similarity once geographic distance is taken into account.

In this study we were investigated the relationship between two distance matrixes based on morphological traits and SSR markers was estimated by Mantel's test (p= 0.076ns) suggesting no significant relationships between two distance coefficient matrixes (Euclidean's or Jaccard's distances) as well as between Rogers-Tanimoto distance and Jaccard's distance (p = 0.003ns) on the other hand Mantel test, suggesting that there are significant correlation between Rogers-Tanimoto and Euclidean distance (0.389**) (Table 13).

Table 13. Associations among the distance matrices by Mantel test

Matrix	Euclidean	Jaccard
Jaccard	0.076^{ns}	
RT	0.389**	0.003^{ns}

** $P < 0.01$; ns - not significant

5.9. Genetic information of SSR markers

Total of1146 polymorphic fragments were amplified between the 40 different red clover accessions using fifteen SSR primer combinations. The number of bands used in plants varies widely from 61 (Nienhuis et al., 1993) to 1205 (Smith et al., 1990). The number of fragments amplified by each SSR primer combination varied from 24 for RCS3681 to 108 for RCS1729 with an average value of 76.4 per primer combination. High variation of amplification products within cultivars were observed from 18 for Kohler, Nfg Mekra, Cremonese to 43 for Teroba, E-86. High variation within cultivars of red clover has also been observed using chloroplast DNA RFLP markers (Milligan 1991). These high levels of variation within populations using molecular markers are consistent with observations on other outcrossing plant species, sueh as alfalfa (Yu and Pauls, 1993b) and perennial ryegras (Sweeney and Danneberger, 1994).

The breeding schemes and the synthetic structure of the cultivars with several parental families maintain a large within cultivar variation, at least for neutral markers, as observed in alfalfa (Flajoulot et al. 2005). We found higher genetic diversity than other studies based on RAPD markers (Kongkiatngam et al., 1995, 1996; Ulloa et al., 2003) and isozyme markers (Mosjidis and Klingler 2006), probably because microsatellite markers display high mutation rates, and are thus expected to reveal fairly high amounts of polymorphisms especially when used at the species level (Thuillet et al., 2005).

All the SSR primers used in the present study produced discrete, scorable and unambiguous bands. The details of SSR primers used for assessing the molecular diversity among 40 red clover accessions are given in table 5.

The PCR product size obtained by the amplification of SSR primers ranged from 105 to 270 bp.

5.10. SSR genetic distance similarity and cluster analysis

The banding pattern of the SSR markers scored in the form of binary data was used for computing Jaccard's similarity index.

Estimates of genetic similarity matrices based on the SSR molecular marker data for all pairwise combinations of the 40 red clover accessions. The similarity coefficients based on 15 SSR markers ranged from 0.50 to 0.90 among the 40 accessions studied. This genetic diversity agreed with previous reports in RAPD markers (Kongkiatngam et al., 1996, Campos-de-Quiroz and Ortega-Klose 2001, Ulloa Ortega and Campos 2003) or wild

populations (Kolliker et al., 2003, Herrmann et al., 2005) of red clover were analyzed. In addition similarity values detected for red clover were comparable to those detected for a forage species like alfalfa, whose Nei and Li's similarity values averaged 0.57 (range 0.46–0.62) (Kidwell et al., 1994) and whose pollination system is similar to that of red clover. The highest similarity index (0.918) was recorded between the genotypes Moser and SA 2. The lowest similarity index (0.520) was recorded by the genotype Cremonese. The similarity values obtained for each pair wise comparison of SSR markers among the 40 red clover accessions were used to construct dendrogram results are presented in (figure 8). The genetic similarity within the 40 accessions was very high, with very few values lower than 0.6 .The dendrogram produced ten distinct clusters (figure 8). The cluster size varied from 14 genotyps of red clover (cluster 1) to 1 (Clusters 9, 10), data on the genetic diversity of red clover population with different geographical origin are presented in this study. The measures of relative genetic distances among populations did not completely correlate with geographical distances of places of their origin.

For instance, NCPGRU1 and Viola Hruszowska with high geographical distances grouped in cluster I together, also E-86, E128 and Aberystwyth populations with enough geographical distance grouped in cluster II. The same results was obtained on alfalfa (Tucak et al., 2008; Touil et al., 2008), *Bunium persicum* (Pezhmanmehr et al., 2010), *Daucus carota* (Bradeen et al., 2002), *Phaseolus vulgaris* (Martins et al., 2006), *Matricaria chamomilla* (Solouki et al., 2008) and Grapevine (Theocharis et al., 2010).

Cluster I comprised of 14 including accessions within further divided into two subcluster. The subcluster I-1 comprised (NCPGRU 1) from Ukrania, two from Poland (Skrzeszowicka , Viola Hruszowska) and (Teroba) from Holand , Subcluster I-2 contained ten accessions, three from Germany (Matri ,Atelo ,Remy) , three from Italia (Valente, Cremonese, Perseo), one from Swedan (Pelly), one from Denmark (Krano), one from Czech Republic (Slovenska B), and one from Ukrania (NCPGRU8).

Cluster II contained three accessions, two from Belgium (E-86 and E128), and one from UK (Aberystwyth).

Cluster III comprised of three accessions Crop (France), E-26 (Bulgaria) and Rozbehy (Slovakia).

Accessions E-97, E-28 that clustered closely together in cluster IV were collected from (Bulgaria)

These accessions clustered closely together with accessions Bombi (Sweden), Nfg Mekra (Germany) and Mir (Russia).

Cluster V include accessions Gkt Junior, Longevo from Hungaria and Italia respectively.

Cluster VI contain seven accessions two from Serbia (Kolubara, NS-Ravanica), Pales from France, Moser from Switzerland, Fanny from Swedan, Jubilatka from Germany and Rotonde Kuhn from Holland.

Cluster VII consist of two accessions NCPGRU 6, from Ukraine and Kenland-Bo from Bolivia.

Cluster VIII comprised of two accessions Kohler, Brezova from Switzerland and Slovakia respectively.

Cluster IX includes one accession Changins from Switzerland.

Cluster X consists of one accession SA-2 from Australia.

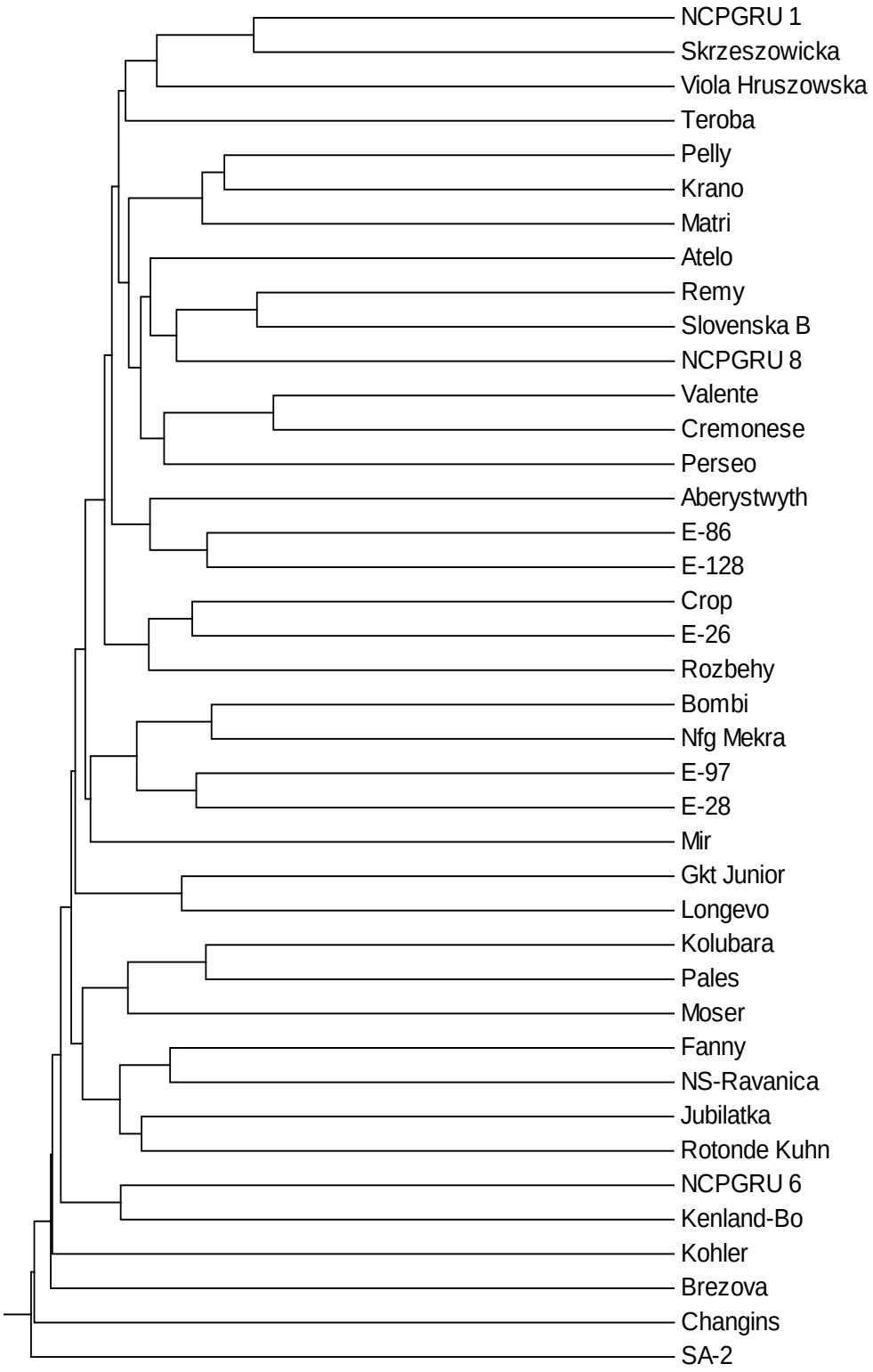

Figure 8. Dendrogram of 40 red clover accessions revealed by UPGMA cluster analysis based on SSR markers data

5.11. Principal coordinates analysis using SSR markers

PCoA was used to identify multidimensional relationships that describe portions of the genetic variance in a data set; the genetic relationships among 40 red clover genotypes were also determined using Principal coordinate analysis PCoA of GD estimates and are presented in (figure 9, 10, 11). According full accessions name, Origin grouping and accessions classifications respectively. the first (PCoA 1) and the second (PCoA 2) principal coordinate axes accounted for 5.5% and 5% of the total variation, respectively. So the first two components of the PCoA between red clover genotypes represented 10.5 % of the variance, therefore PCoA was useful for graphical representation, as it adequately summarizes the microsatellite data. Two axes separated the red clover genotypes into six groups. PCoA grouped accessions Kolubara, Fanny, Jubilatka and Pales together in first group also clustered in VI of the dendrogram (figure 8). Accessions NS-Ravanica, Teroba, E-97, Kohler, perseo, Atelo, E-26, NCPGRU6, and Brezova clustered together in second group and separated in dendrogram. The PCoA biplot (figure 9) clustered accessions similarly to the dendrogram based on their genetic similarity with some differences. Accessions grouping together, in cluster I of the dendrogram (figure 8) also grouped together in the third cluster. In biplot with some exceptions. Accessions Cremonese and Slovenska B clustered together in the dendrogram, but in principal coordinate analysis (PCoA) was separated from the group and clustered together with other group, this might be due to the pedigree relationships with the rest of the accessions in cluster I of the dendrogram accessions Cremonese and Slovenska B clustered together in fourth group with accessions Moser, Changins, Nfg Mekra,Mir, Bombi, , E-128, Longevo,and E-86 , all collected from the center and east of Europe.

Accessions, E-128, Crop, E-26 and Rozbehi clustered together in cluster II of the dendrogram (figure 8) also grouped together in the biplot.

PCoA grouped accessions Changins, Nfg Mekra, Remy, Mir, Aberystwyth, Viola, E-28, Gkt Junior, NCPGRU 8, in fifth group and separated in dendrogram.

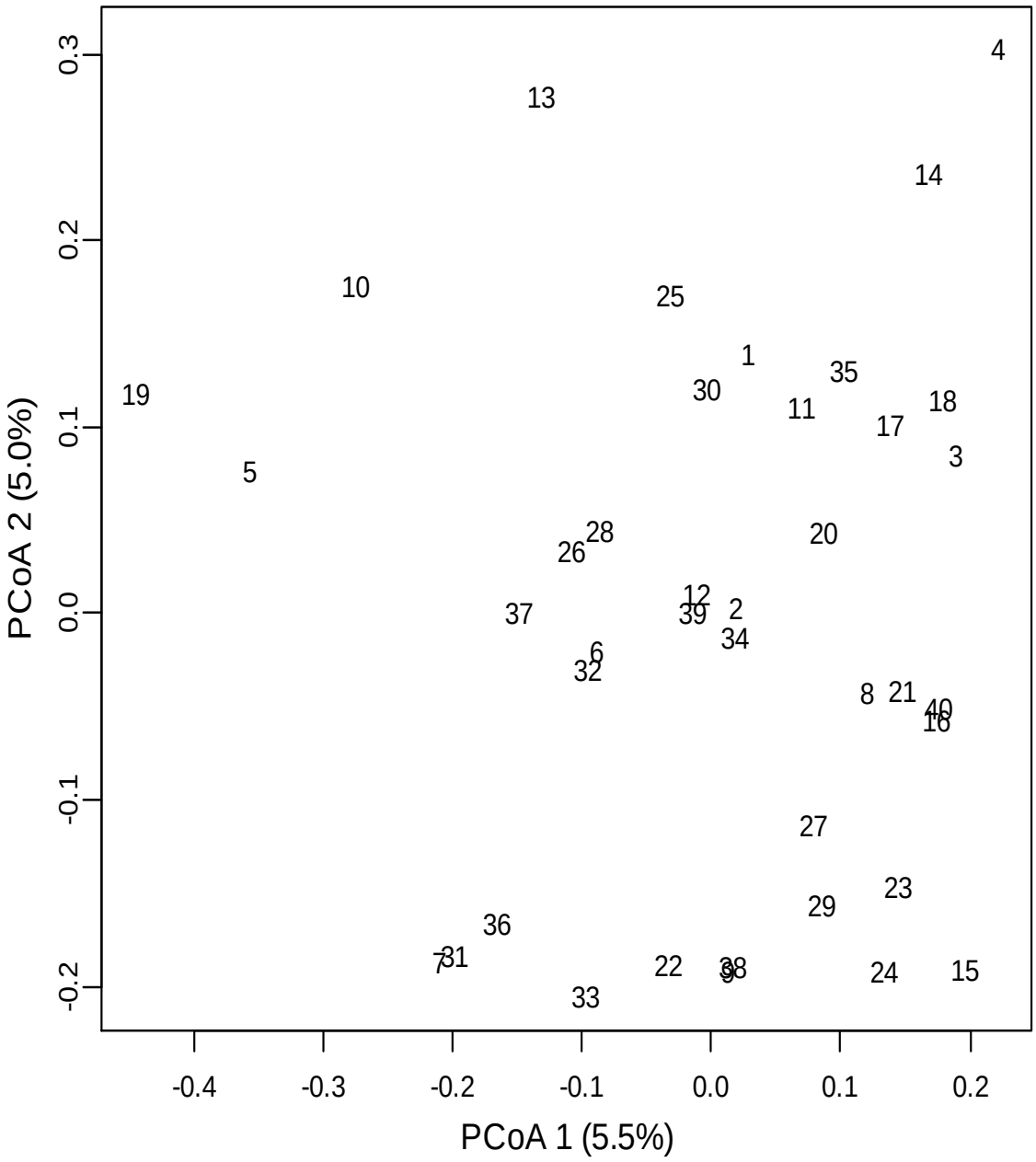

Figure 9. First and second Principal Coordinate Analysis (PCoA), (Origin grouping) the number is genotype name presented in Table 1.

In sixth group accessions SA-2 and Matri separated in dendrogram but clustered together in PCoA. The Principal Coordinate Analysis (PCoA) separated accessions better than the dendrogram based on the genetic similarity analysis and geographical location. PCoA provided a better diversity structure than the dendrogram since PCoA used three dimensions, compared to one dimension for the dendrogram. The PCoA also generated a good separation of country grouping and genotype classifications.

In addition principal coordinate analysis (PCoA) was used to analysis of red clover

accessions according country groups (figur 10).

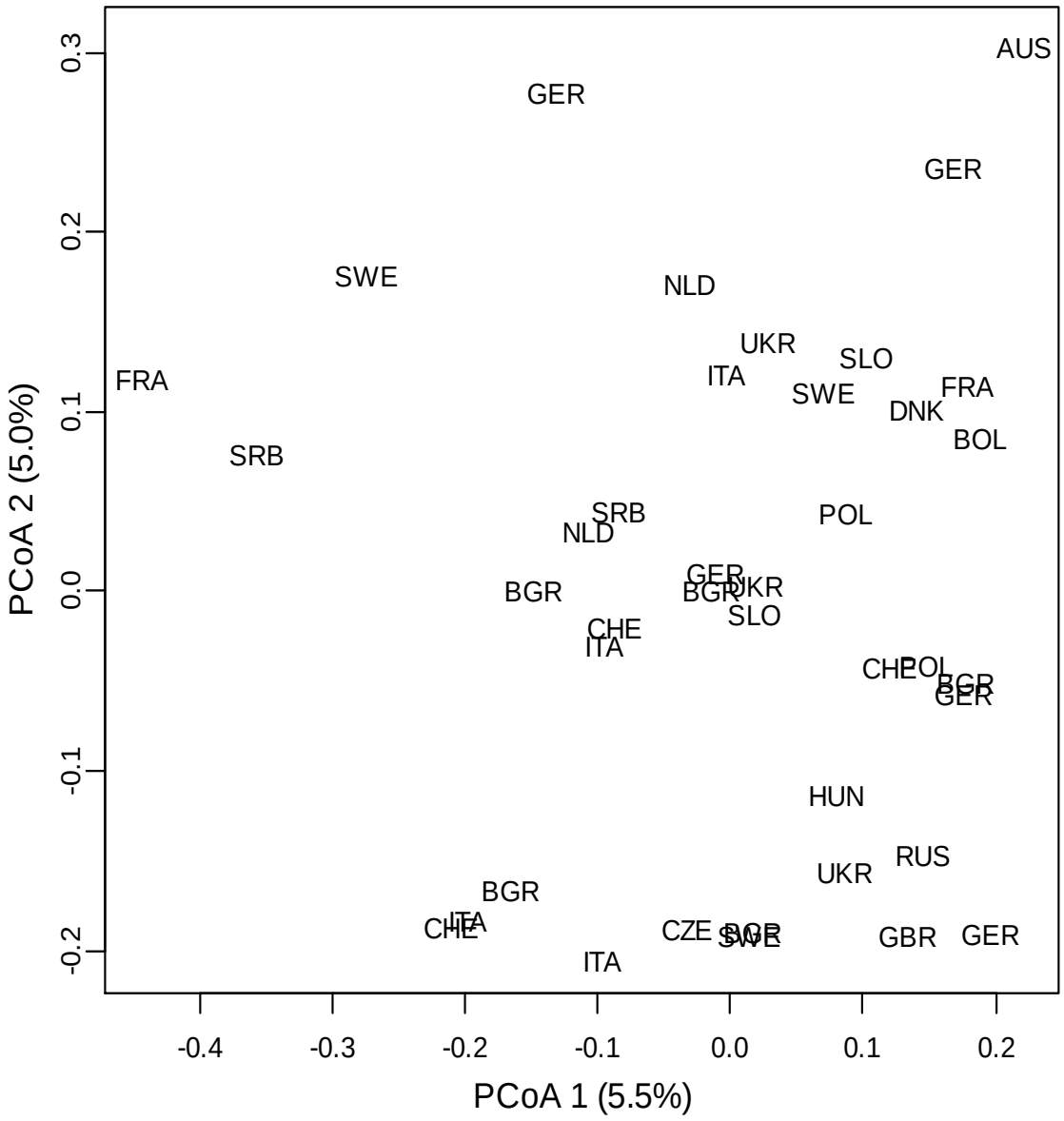

Figure 10. First and second principal coordinate analysis (PCoA) (country grouping) the number is genotype name presented in Table 1.

The analysis revealed distinct clustering for accessions in six groups, accessions origin shown to a varying extent overlap.

And no clearcut relation between genetic diversity assessed by SSR and geographical origin could be established in the present study, same as reported by (Dias et al., 2008) for *T. pratense.* In *Trifolium repens* (Zhang et al., 2010).

Based on accessions classifications (status of accessions) populations dispersed in five groups (2, 3, 4, 5, 6) (figure11) and ploidy level scattered the tetraploid in all four groups (1-2-3-6) (figure 11) with overlap with diploid.

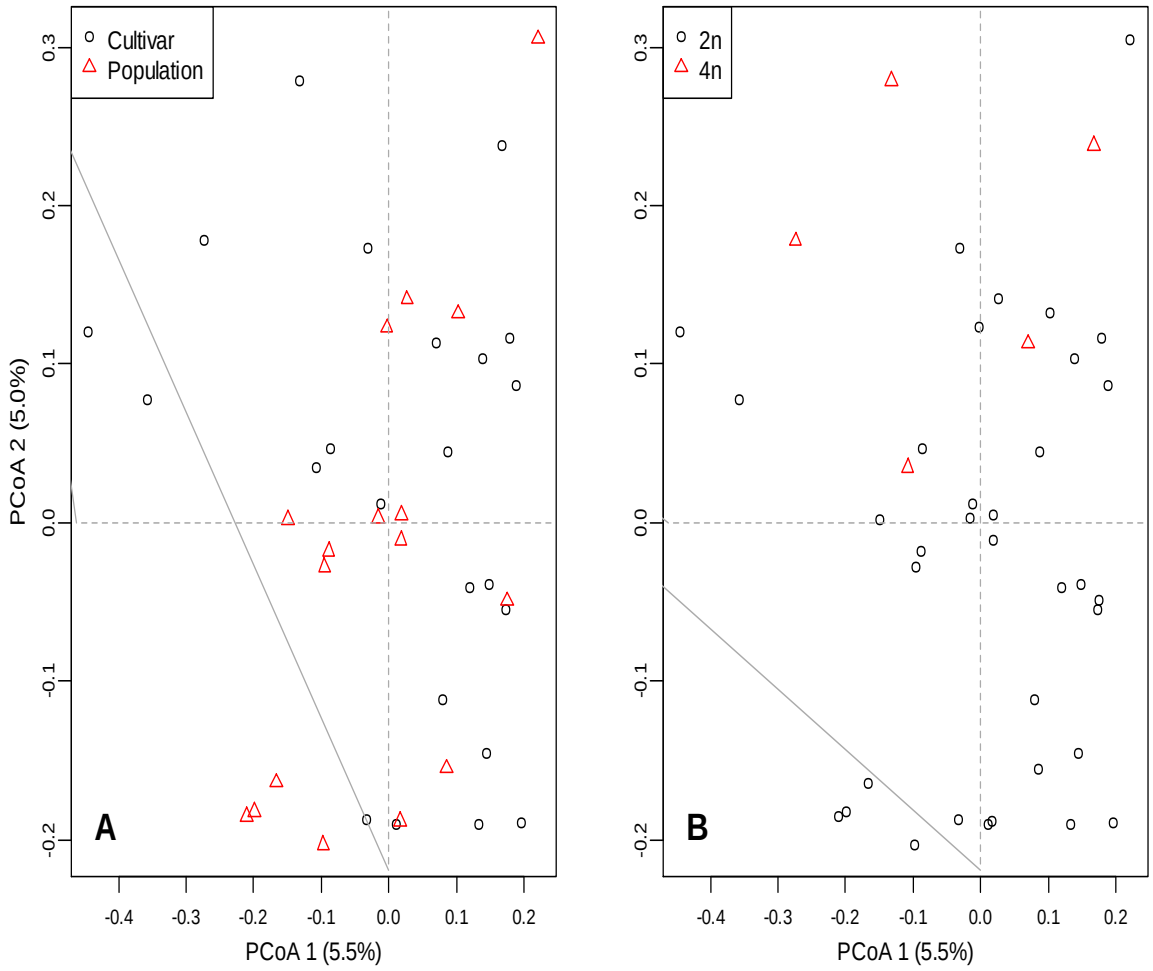

Figure11. First and second principal coordinate analysis (PCoA) using accessions classifications.

A. status of accessions **B.** Ploidy level of accessions.

5.12. Analysis of molecular variance (AMOVA)

The AMOVA (Analysis of Molecular Variance) using the SSR data according level of polyploidy revealed that the total variation observed was high and accounted for 99.91% between groups and 0.09% was observed among groups in the present study (Table14). The overall (*F*ST = 0.0009; *P* =0.449) demonstrated the existence of high genetic differentiation among red clover accessions (Table14).

The AMOVA analysis using SSR molecular marker revealed most of the variation was partitioned between red clover groups than among groups.

Table14. Analysis of molecular variance (AMOVA)

Source of variation	**df**	**Variance component**	**Percentage of variation**	F_{ST}	***P***
		Level of polyploidy			
Among groups	1	0.00036	0.09	0.00092	0.449
Between groups	38	0.38454	99.91		
Total	39	0.38490			
		Status of cultivar			
Among groups	1	0.00226	0.59	0.00585	0.137
Between groups	38	0.38350	99.41		
Total	39	0.38576			

The total amount of genetic variation was also partitioned by AMOVA into components according to the Status of cultivar. Based upon the analysis of the accessions structure, the AMOVA results (Table14) was observed significant variation between groups component accounts for 99.41% of the total variance and the remnant amounts of the total genetic variation was found among groups 0.59 %. In amount of the genetic variation and (*F*ST = 0.00585; *P* =0.137).

Other studies in red clover (Kolliker et al., 2003) and in other forage crops (Kolliker et al., 2001) found an extensive within population genetic diversity also when using different types of molecular markers (Campos-de-Quiroz and Ortega-Klose 2001; Ulloa et al., 2003; Kolliker et al., 2003). The variation for within-population diversity ranged from 68.3 to 99.5% in studies realized with SSR markers in other allogamous legume species such as alfalfa (Mengoni et al., 2000) and in red clover using AFLPs (Herrmann et al., 2005) and RAPDs (Ulloa et al., 2003).

In addition analysis of the seed yield of 11 *Medicago sativa* L. populations showed that the among-population variance accounted for 5% to 31% of the total genetic variance for seed yield components, while the within-population variance explained 69% to 95% (Bolanos-Aguilar et al., 2000).

6. Conclusions

A total of 40 red clover accessions were evaluated for eight quantitative and five qualitative traits to determine the extent of phenotypic diversity. The ANOVA identified the relative importance of each of the quantitative traits. The traits substantially contributed differentiating the accessions studied. Moreover, the simple correlations between each pair of quantitative characters recorded, clearly depicted the close association between some traits.

High genetic distances were observed among some accessions and it is an important parameter in selecting parents for breeding.

Cluster analysis grouped the accessions into six basic groups. All the accessions were distinctly separated from each other and the accessions with similar morphological characters grouped together. Phenotypic variation for five qualitative traits was estimated using the Shannon-Weaver diversity index. The diversity values for the characters showed narrow variability among them. Based on the observed variation both for quantitative and qualitative traits, it could be concluded that studying the phenotypic diversity among red clover accessions is important to identify the genetic potential of parental lines and increase the efficiency of the red clover breeding programmers. Thus all the accessions are phenotypically variable and phenotypic markers can be used to distinguish accessions.

SSR markers appears to be an effective approach in resolving genetic variation in red clover, grouping divergent germplasm and assessing the effects of breeding in terms of genetic variability. This information could be used for a better informed decision process regarding the selection of parents for developing new synthetic populations and the selection of divergent pairs of parents which could be used to generate linkage maps in this species. Nevertheless, before drawing definite conclusions further analyses are required. The incorporation of marker technology for traits difficult to assess will represent a major advance in the genetic improvement of red clover.

Understanding the diversity of red clover germplasm collections is important for effective exploitation of their genetic potential as well as for selection of genotypes as breeding lines, maintenance and for conservation. The SSR marker analysis has successfully provided a precise estimation of genetic diversity among tested red clover accessions. DNA markers cover the whole genome while morphological markers cover

only selected traits in breeding programmes and DNA markers are therefore more reliable.

SSR marker data identified and clustered accessions mainly according to their collection sites. The PCoA provided a similar structure but with some differences to that of the dendrogram's clustering patterns, suggesting the effectiveness of PCoA analysis in genetic diversity analysis. In future, more primer combinations should be included for genetic analysis in red clover germplasm. Therefore, further investigations could involve a larger number of samples and more primers with a wider range of collection sites for accessions within regions including other regions using different DNA-based molecular markers analysis.

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APPENDICES

Appendix 1.Student-Newman-Keuls Test for Number of Internodes (NOI)

Means with the same letter are not significantly different

SNK Grouping	Mean	N	genotype
A	6.6333	3	33
A	6.5967	3	2
B A	6.2800	3	10
B A C	6.0867	3	20
B D A C	5.9767	3	25
B D A C	5.9233	3	15
B D A C	5.8600	3	16
B D A C	5.8533	3	23
B D A C	5.8167	3	18
B D A C	5.8100	3	17
E B D A C	5.6700	3	14
E B D A C	5.6267	3	4
E B D A C F	5.5767	3	21
E B D A C F	5.5100	3	27
E B D A C F	5.5100	3	24
E B D A C F	5.5033	3	5
E B D A C F	5.4200	3	3
E B D A C F	5.3900	3	29
E B D A C F	5.3000	3	11
E B D A C F	5.2967	3	32
E B D A C F	5.2833	3	30
E B D A C F	5.2700	3	12

E	B	D	A	C	F	5.2567	3	9
E	B	D	A	C	F	5.2367	3	7
E	B	D	A	C	F	5.1967	3	19
E	B	D	A	C	F	5.1900	3	26
E	B	D	A	C	F	5.1800	3	1
E	B	D	A	C	F	5.1767	3	22
E	B	D	A	C	F	5.1100	3	13
E	B	D	A	C	F	5.0667	3	8
E	B	D	A	C	F	5.0400	3	6
E	B	D		C	F	4.9133	3	31
E	B	D		C	F	4.8100	3	28
E	B	D		C	F	4.7000	3	34
E	B	D		C	F	4.6667	3	37
E		D		C	F	4.5000	3	38
E		D		C	F	4.4667	3	40
E		D			F	4.4000	3	35
E					F	4.1333	3	36
					F	4.0000	3	39

See table 1 for genotype number

Appendix 2.Student-Newman-Keuls Test for Number of Braches (NOB)

Means with the same letter are not significantly different

SNK Grouping	Mean	N	genotype
A	5.5967	3	2
A	5.5933	3	33
B A	5.3200	3	10
B A C	5.1167	3	20
B D A C	4.9233	3	15
B D A C	4.9100	3	23
B D A C	4.8900	3	25
B D A C	4.8867	3	16
B D A C	4.8733	3	17
E B D A C	4.6667	3	37
E B D A C F	4.6533	3	4
E B D A C F	4.6300	3	14
E B D A C F	4.5667	3	21
E B D A C F	4.5100	3	24
E B D A C F	4.5100	3	27
E B D A C F	4.4967	3	5
E B D A C F	4.4167	3	29
E B D A C F	4.3833	3	3
E B D A C F	4.3333	3	11
E B D A C F	4.2767	3	12
E B D A C F	4.2733	3	18
E B D A C F	4.2667	3	32

E	B	D	A	C	F	4.2567	3	9
E	B	D	A	C	F	4.2433	3	30
E	B	D	A	C	F	4.2333	3	1
E	B	D	A	C	F	4.2267	3	7
E	B	D	A	C	F	4.2167	3	26
E	B	D	A	C	F	4.1800	3	19
E	B	D	A	C	F	4.1700	3	22
E	B	D	A	C	F	4.1100	3	13
E	B	D	A	C	F	4.0667	3	8
E	B	D	A	C	F	4.0400	3	6
E	B	D		C	F	3.9133	3	31
E	B	D		C	F	3.8333	3	34
E	B	D		C	F	3.8167	3	28
E	B	D		C	F	3.6667	3	38
E		D		C	F	3.5667	3	40
E		D			F	3.4000	3	35
E					F	3.1333	3	36
					F	3.0000	3	39

Appendix 3.Student-Newman-Keuls Test for Stem Length (STL)

Means with the same letter are not significantly different

SNK Grouping		Mean	N	genotype
	A	50.700	3	2
	A	50.633	3	3
B	A	49.200	3	20
B	A	48.833	3	10
B	A	48.700	3	33
B	A	48.067	3	1
B	A	47.833	3	12
B	A	47.767	3	13
B	A	47.167	3	19
B	A	46.900	3	5
B	A	46.800	3	25
B	A	46.733	3	14
B	A	46.600	3	15
B	A	46.267	3	18
B	A	46.233	3	32
B	A	45.800	3	7
B	A	45.733	3	11
B	A	45.633	3	4
B	A	45.567	3	21
B	A	45.400	3	16
B	A	45.300	3	9
B	A	45.300	3	29

B	A	44.800	3	17
B	A	44.633	3	30
B	A	44.400	3	27
B	A	44.000	3	8
B	A	43.733	3	22
B	A	43.467	3	6
B	A	43.400	3	23
B	A C	42.200	3	28
B	A C	41.933	3	24
B D	A C	39.667	3	26
B D	A C	38.967	3	31
B D	A C	38.567	3	40
B D	E C	36.633	3	34
F D	E C	32.233	3	35
F D	E C	31.667	3	37
F D	E	30.600	3	36
F	E	28.000	3	39
F		26.500	3	38

Appendix 4.Student-Newman-Keuls Test for Stem Thickness diameter (STD)

Means with the same letter are not significantly different

SNK Grouping	Mean	N	genotype
A	5.3500	3	10
B A	5.1200	3	1
B A C	4.9933	3	13
B D A C	4.9733	3	14
E B D A C	4.6900	3	25
E B D A C	4.6867	3	11
E B D A C F	4.5900	3	32
E B D A C F	4.5733	3	3
E B D A C F	4.5400	3	17
E B D A C F	4.4300	3	12
E B D A C F	4.4167	3	26
E B D A C F	4.4067	3	15
E B D A C F	4.3667	3	23
E B D A C F	4.3367	3	27
E B D A C F	4.2633	3	2
E B D A C F	4.2067	3	9
E B D A C F	4.1900	3	33
E B D A C F	4.1800	3	4
E B D A C F	4.0767	3	6
E B D A C F	4.0600	3	30
E B D A C F	3.9967	3	28
E B D A C F	3.9900	3	18

E	B	D	A	C	F	3.9733	3	29
E	B	D	A	C	F	3.9367	3	16
E	B	D	A	C	F	3.9033	3	20
E	B	D	A	C	F	3.8933	3	19
E	B	D	A	C	F	3.8100	3	22
E	B	D	A	C	F	3.7267	3	31
E	B	D	A	C	F	3.6967	3	24
E	B	D	A	C	F	3.6900	3	21
E	B	D	A	C	F	3.6333	3	40
E	B	D	A	C	F	3.5167	3	37
E	B	D		C	F	3.5000	3	8
E	B	D		C	F	3.3467	3	7
E	B	D		C	F	3.2667	3	36
E		D		C	F	3.2333	3	34
E		D		C	F	3.1933	3	35
E		D			F	3.1100	3	5
E					F	2.8833	3	39
					F	2.7833	3	38

Appendix 5 .Student-Newman-Keuls Test for Medium Lamina Length (MLL)

Means with the same letter are not significantly different

SNK Grouping	Mean	N	genotype
A	36.233	3	3
A	35.800	3	1
B A	34.433	3	10
B A	34.067	3	2
B A	33.867	3	19
B A C	33.433	3	13
B A C	33.300	3	9
B A C	33.000	3	12
B A C	33.000	3	14
B A C	32.767	3	29
B A C	32.700	3	18
B A C	32.700	3	7
B A C	32.667	3	8
B A C	32.667	3	22
B A C	32.600	3	21
B A C	32.567	3	6
B A C	32.433	3	25
B A C	32.433	3	20
B D A C	32.033	3	15
B D A C	32.033	3	11
B D A C	31.900	3	28
B D A C	31.900	3	5

	B	D	A	C	31.633	3	30
	B	D	A	C	31.533	3	16
	B	D	A	C	31.467	3	27
	B	D	A	C	31.467	3	32
	B	D	A	C	31.233	3	4
E	B	D	A	C	29.833	3	40
E	B	D	A	C	29.133	3	31
E	B	D	A	C	28.833	3	23
E	B	D	A	C	28.633	3	33
E	B	D	A	C	28.467	3	26
E	B	D	A	C	28.100	3	17
E	B	D	A	C	27.900	3	24
E	B	D		C	25.333	3	34
E	B	D		C	25.333	3	36
E		D		C	24.567	3	38
E		D			23.700	3	39
E		D			23.600	3	35
E					23.167	3	37

Appendix 6.Student-Newman-Keuls Test for Medium Lamina diameter (MLD)

Means with the same letter are not significantly different

SNK Grouping	Mean	N	genotype
A	20.233	3	3
B A	17.967	3	10
B A C	17.300	3	13
B D A C	17.033	3	1
E B D A C	16.667	3	4
E B D A C F	16.567	3	14
E B D G C F	16.033	3	11
E B D G H C F	15.633	3	26
E B D G H C F	15.467	3	32
E B D G H C F	15.300	3	9
E B D G H C F	15.133	3	40
E B D G H C F	15.000	3	25
E B D G H C F	14.867	3	12
E B D G H C F	14.533	3	2
E B D G H C F	14.467	3	6
E B D G H C F	14.367	3	28
E B D G H C F	14.333	3	23
E B D G H C F	14.333	3	21
E B D G H C F	14.333	3	30
E B D G H C F	14.233	3	19
E B D G H C F	14.233	3	8
E B D G H C F	14.200	3	7

E	B	D	G	H	C	F	13.900	3	29
E	B	D	G	H	C	F	13.767	3	16
E	B	D	G	H	C	F	13.667	3	18
E	B	D	G	H	C	F	13.500	3	27
E	B	D	G	H	C	F	13.400	3	17
E	B	D	G	H	C	F	13.367	3	31
E	B	D	G	H	C	F	13.333	3	15
E	B	D	G	H	C	F	13.267	3	22
E	B	D	G	H	C	F	13.200	3	20
E	B	D	G	H	C	F	13.167	3	5
E	B	D	G	H	C	F	13.100	3	33
E		D	G	H	C	F	12.333	3	36
E		D	G	H		F	11.967	3	24
E			G	H		F	11.767	3	35
			G	H		F	11.433	3	39
			G	H			11.300	3	38
				H			10.767	3	34
				H			10.533	3	37

Appendix 7.Student-Newman-Keuls Test for Yield of Green Mass (YGM)

Means with the same letter are not significantly different

SNK Grouping	Mean	N	genotype
A	337.00	3	10
B A	301.27	3	9
B A C	289.87	3	23
B D A C	276.27	3	17
E B D A C	266.27	3	1
E B D A C F	232.00	3	11
E B D A C F	217.77	3	2
E B D G C F	193.40	3	20
E B D G C F	192.90	3	32
E B D G C F	189.60	3	4
E B D G C F	189.10	3	21
E B D G C F	179.40	3	33
E B D G C F	178.00	3	3
E B D G C F	177.83	3	25
E B D G C F	173.47	3	14
E B D G C F	172.53	3	27
E D G C F	157.53	3	15
E D G C F	154.33	3	26
E D G C F	150.77	3	24
E D G F	143.03	3	5
E D G F	142.77	3	16
E D G F	139.37	3	19

	E	D	G	F	138.60	3	30
	E	D	G	F	138.53	3	13
	E	D	G	F	137.80	3	12
	E	D	G	F	134.83	3	7
	E	D	G	F	133.70	3	8
	E	D	G	F	125.17	3	22
	E	D	G	F	124.67	3	29
	E	D	G	F	124.30	3	18
	E		G	F	119.50	3	6
	E		G	F	118.80	3	28
			G	F	109.53	3	40
			G	F	80.83	3	37
			G	F	80.00	3	39
			G	F	79.00	3	31
			G	F	75.57	3	36
			G		53.33	3	38
			G		48.33	3	34
			G		39.73	3	35

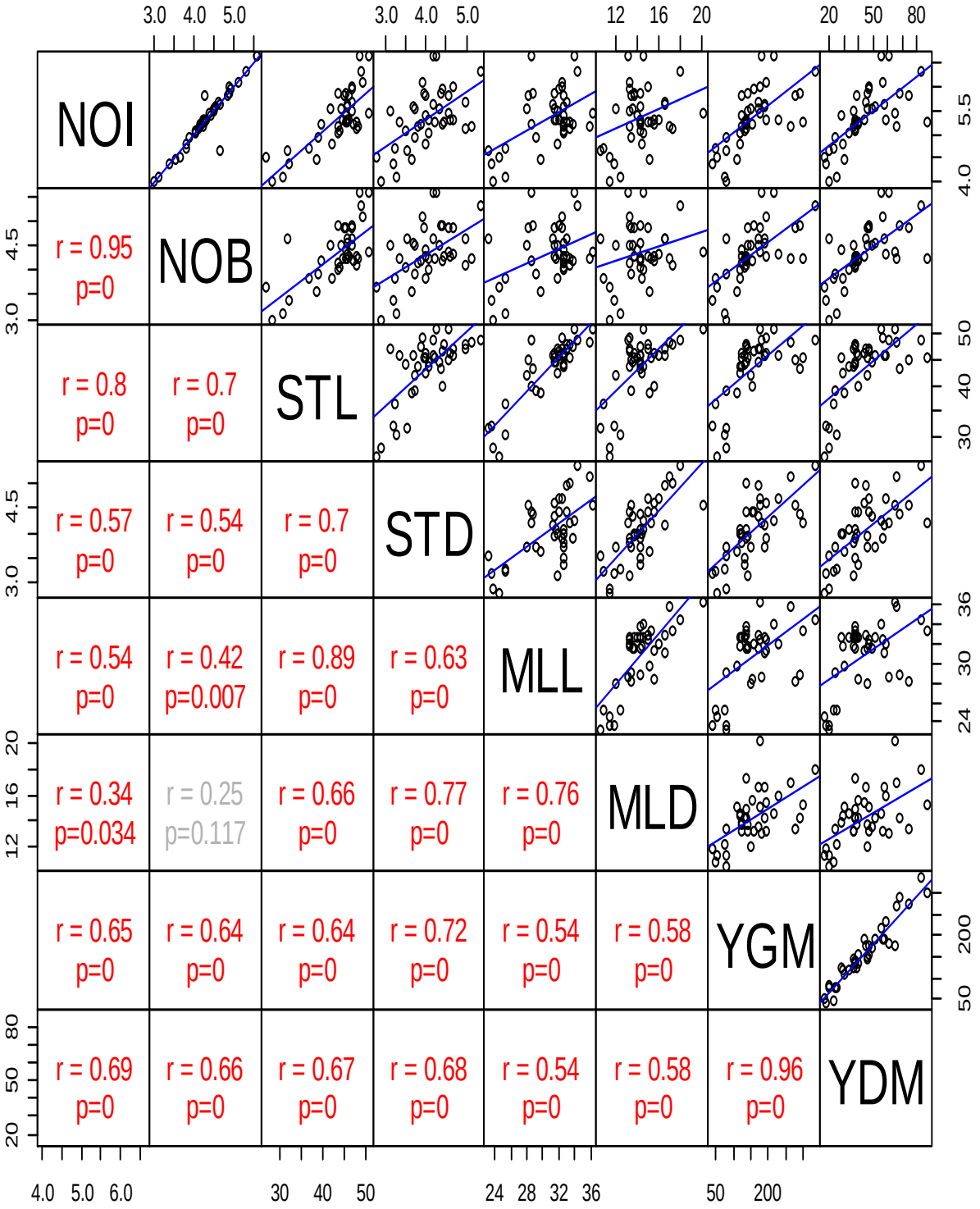
Appendix 8.Student-Newman-Keuls Test for Yield of Dry matter (YDM)

Means with the same letter are not significantly different

SNK Grouping	Mean	N	genotype
A	87.10	3	9
B A	83.10	3	10
B A C	73.97	3	17
B D A C	68.57	3	23
E B D A C	66.30	3	1
E B D A C F	64.90	3	3
E B D A G C F	60.97	3	33
E B D H A G C F	58.80	3	11
E B I D H A G C F	57.30	3	4
E J B I D H A G C F	56.43	3	20
E J B I D H A G C F	56.03	3	2
E J B I D H G C F	51.27	3	21
E J B I D H G C F	49.33	3	27
E J I D H G C F	46.73	3	15
E J I D H G C F	46.70	3	25
E J I D H G C F	46.43	3	16
E J I D H G C F	45.70	3	24
E J I D H G C F	45.27	3	14
E J I D H G C F	44.17	3	32
E J I D H G C F	39.70	3	26
E J I D H G C F	39.43	3	7
E J I D H G C F	38.50	3	18

E	J	I	D	H	G	C	F	38.23	3	12
E	J	I	D	H	G	C	F	38.07	3	19
E	J	I	D	H	G	C	F	38.00	3	30
E	J	I	D	H	G	C	F	37.40	3	13
E	J	I	D	H	G	C	F	37.37	3	8
E	J	I	D	H	G	C	F	37.03	3	22
E	J	I	D	H	G	C	F	36.73	3	5
E	J	I	D	H	G		F	33.20	3	6
E	J	I	D	H	G		F	29.90	3	40
E	J	I	D	H	G		F	29.50	3	28
E	J	I		H	G		F	28.13	3	29
	J	I		H	G		F	25.60	3	36
	J	I		H	G			24.50	3	31
	J	I		H	G			22.80	3	34
	J	I		H				19.93	3	39
	J	I		H				19.83	3	37
	J	I						18.00	3	35
	J							16.43	3	38

Appendix 9. Associations among the distance matrices by Mantel test



Appendix 10. Estimates of genetic distance based on phenotypic characters for all pair-wise comparisons of 40 red clover accessions

GE .No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	0.00																																								
2	4.0	0.00																																							
3	2.3	4.2	0.00																																						
4	2.6	2.8	2.6	0.00																																					
5	4.7	3.7	4.8	2.8	0.00																																				
6	3.7	4.4	4.0	2.5	2.1	0.00																																			
7	4.1	4.0	4.2	2.4	0.9	2.4	0.00																																		
8	4.1	4.3	4.2	4.6	1.4	1.0	0.5	0.00																																	
9	2.3	4.0	3.6	2.6	4.3	4.1	3.9	4.0	0.00																																
10	3.0	3.4	3.8	3.8	6.2	6.0	6.0	6.1	3.5	0.00																															
11	1.6	3.4	2.7	1.3	3.5	2.6	3.0	2.9	2.1	3.5	0.00																														
12	3.0	3.6	3.5	1.9	2.4	1.1	1.8	1.7	3.7	5.1	2.0	0.00																													
13	2.6	4.5	2.6	2.4	3.8	2.2	3.1	2.9	4.0	4.8	2.1	1.5	0.00																												
14	2.2	2.9	2.5	1.5	3.6	2.6	3.1	3.1	3.4	3.7	1.5	1.6	1.5	0.00																											
15	3.5	2.2	4.1	2.0	2.4	2.5	2.5	2.6	3.6	4.5	2.4	1.8	3.0	2.6	0.00																										
16	3.9	2.5	4.1	1.8	1.7	2.2	1.9	2.1	3.7	4.9	2.6	1.9	3.2	2.3	0.8	0.00																									
17	3.5	3.1	4.6	2.5	3.9	4.1	4.0	4.1	2.5	3.6	2.4	3.6	4.3	3.2	2.6	2.8	0.00																								
18	3.8	3.3	4.1	2.2	1.6	1.5	1.4	1.6	4.0	5.4	2.7	1.3	2.7	2.4	1.5	1.2	3.5	0.00																							
19	3.5	3.8	3.7	2.3	1.6	0.9	0.9	0.9	3.7	5.6	2.4	1.0	2.3	2.5	2.1	1.8	3.8	1.1	0.00																						
20	3.8	1.6	4.2	2.2	2.4	3.2	2.7	3.0	3.3	4.3	2.8	2.6	3.8	2.8	1.2	1.3	2.4	2.1	2.6	0.00																					
21	3.3	2.8	3.6	1.5	1.5	2.0	1.4	1.7	2.9	4.7	2.0	1.8	3.0	2.4	1.6	1.1	2.7	1.4	1.5	1.5	0.00																				
22	4.0	4.1	4.4	2.6	1.5	0.8	0.9	0.7	4.0	6.0	2.8	1.4	2.9	2.9	2.2	1.8	3.9	1.2	0.8	2.8	1.6	0.00																			
23	3.3	2.9	4.3	2.2	3.8	3.9	3.8	3.9	2.3	3.4	2.2	3.4	4.1	3.0	2.5	2.7	0.7	3.4	3.7	2.3	2.5	3.7	0.00																		
24	4.8	4.0	5.3	2.8	1.9	2.4	2.1	2.2	4.1	6.0	3.3	2.7	4.0	3.6	2.2	1.8	3.0	2.0	2.4	2.5	2.0	1.9	3.0	0.00																	
25	3.6	2.0	3.3	1.5	3.0	2.7	2.9	3.0	3.4	3.7	1.9	1.8	2.5	1.1	1.0	1.5	2.6	1.9	2.4	1.7	1.9	2.7	2.4	2.9	0.00																
26	3.6	4.4	4.0	2.1	3.0	1.7	2.5	2.2	3.8	5.4	2.2	1.9	2.3	2.3	2.6	2.4	3.3	2.3	2.3	3.4	2.4	2.1	3.1	2.3	2.5	0.00															
27	3.1	3.0	3.9	1.7	2.2	1.8	2.0	2.0	3.1	4.7	1.8	1.4	2.6	2.0	1.1	1.2	2.4	1.3	1.6	1.8	1.2	1.5	2.3	1.7	1.4	1.8	0.00														
28	4.1	5.0	4.4	3.0	2.4	0.6	1.7	1.1	4.4	6.5	3.0	1.7	2.5	3.1	3.1	2.8	4.5	2.0	1.4	3.8	2.5	1.2	4.3	2.6	3.3	1.8	2.3	0.00													
29	4.0	3.7	4.2	2.4	1.6	1.0	1.3	1.2	4.3	5.8	2.8	1.2	2.6	2.5	1.8	1.6	3.9	0.9	0.9	2.6	1.7	0.8	3.7	2.1	2.2	2.1	1.6	1.5	0.00												
30	3.5	3.7	3.9	2.0	1.8	0.7	1.2	1.0	3.7	5.5	2.2	0.9	2.3	2.2	1.8	1.5	3.4	1.0	0.8	2.6	1.4	0.7	3.3	1.9	2.1	1.5	1.1	1.2	0.8	0.00											
31	5.3	5.5	5.5	3.7	2.6	1.7	2.2	1.8	5.3	7.3	4.0	2.7	3.6	4.0	3.5	3.0	4.9	2.5	2.4	4.2	3.1	1.7	4.8	2.3	3.9	2.2	2.8	1.3	2.0	1.9	0.00										
32	2.4	3.5	3.1	1.4	2.8	1.7	2.3	2.2	3.0	4.4	1.0	1.1	1.6	1.3	1.9	2.1	2.8	1.9	1.7	2.7	1.8	2.0	2.7	2.7	1.6	1.5	1.3	2.2	1.9	1.3	3.1	0.00									
33	4.9	1.9	5.1	3.0	3.6	4.5	4.1	4.4	4.4	4.4	3.7	3.8	4.8	3.5	2.1	2.3	2.6	3.3	4.1	1.7	3.0	4.1	2.6	3.2	2.5	4.1	2.9	5.0	3.8	3.7	5.1	3.7	0.00								
34	7.0	6.9	7.4	5.3	3.7	3.6	3.7	3.5	6.6	8.9	5.6	4.5	5.6	5.8	4.9	4.4	5.9	4.1	4.1	5.4	4.6	3.4	5.9	3.0	5.5	3.9	4.3	3.2	3.8	3.7	2.0	4.8	6.0	0.00						
35	7.7	7.9	7.9	6.1	4.7	4.3	4.6	4.3	7.3	9.6	6.3	5.3	6.1	6.5	5.9	5.5	6.7	5.1	5.0	6.5	5.5	4.3	6.7	4.1	6.4	4.4	5.2	3.8	4.7	4.6	2.7	5.5	7.1	1.4	0.00					
36	7.2	8.0	7.6	5.9	4.8	4.1	4.5	4.1	6.8	9.4	6.0	5.2	5.9	6.4	6.0	5.5	6.6	5.1	4.8	6.6	5.4	4.2	6.5	4.2	6.4	4.2	5.1	3.6	4.7	4.5	2.7	5.3	7.3	2.0	1.1	0.00				
37	7.4	6.8	7.9	5.6	4.3	4.3	4.5	4.3	6.9	8.8	5.9	5.1	6.1	6.0	5.0	4.6	5.7	4.7	4.8	5.6	5.0	4.1	5.7	3.2	5.6	4.1	4.6	4.1	4.3	4.3	2.9	5.2	5.8	1.9	2.4	3.0	0.00			
38	8.1	8.1	8.4	6.5	5.0	4.8	4.9	4.6	7.6	10.0	6.8	5.9	6.8	7.0	6.3	5.7	7.0	5.5	5.4	6.8	5.8	4.7	7.0	4.4	6.8	4.9	5.6	4.4	5.1	5.0	3.2	6.1	7.4	1.9	1.3	1.7	2.4	0.00		
39	8.2	8.8	8.6	6.9	5.6	5.1	5.3	5.0	7.7	10.3	6.9	6.2	6.9	7.4	6.9	6.4	7.4	6.0	5.8	7.4	6.2	5.1	7.3	5.0	7.3	5.2	6.1	4.6	5.6	5.4	3.7	6.3	8.1	2.5	1.4	1.1	3.3	1.5	0.00	
40	4.9	6.0	5.1	3.7	3.1	1.9	2.4	1.9	4.9	7.3	3.8	2.9	3.4	4.1	4.1	3.7	5.1	3.1	2.6	4.8	3.3	2.2	4.9	3.1	4.3	2.3	3.3	1.3	2.7	2.3	1.4	3.1	5.8	2.9	3.0	2.6	3.8	3.6	3.6	0.0

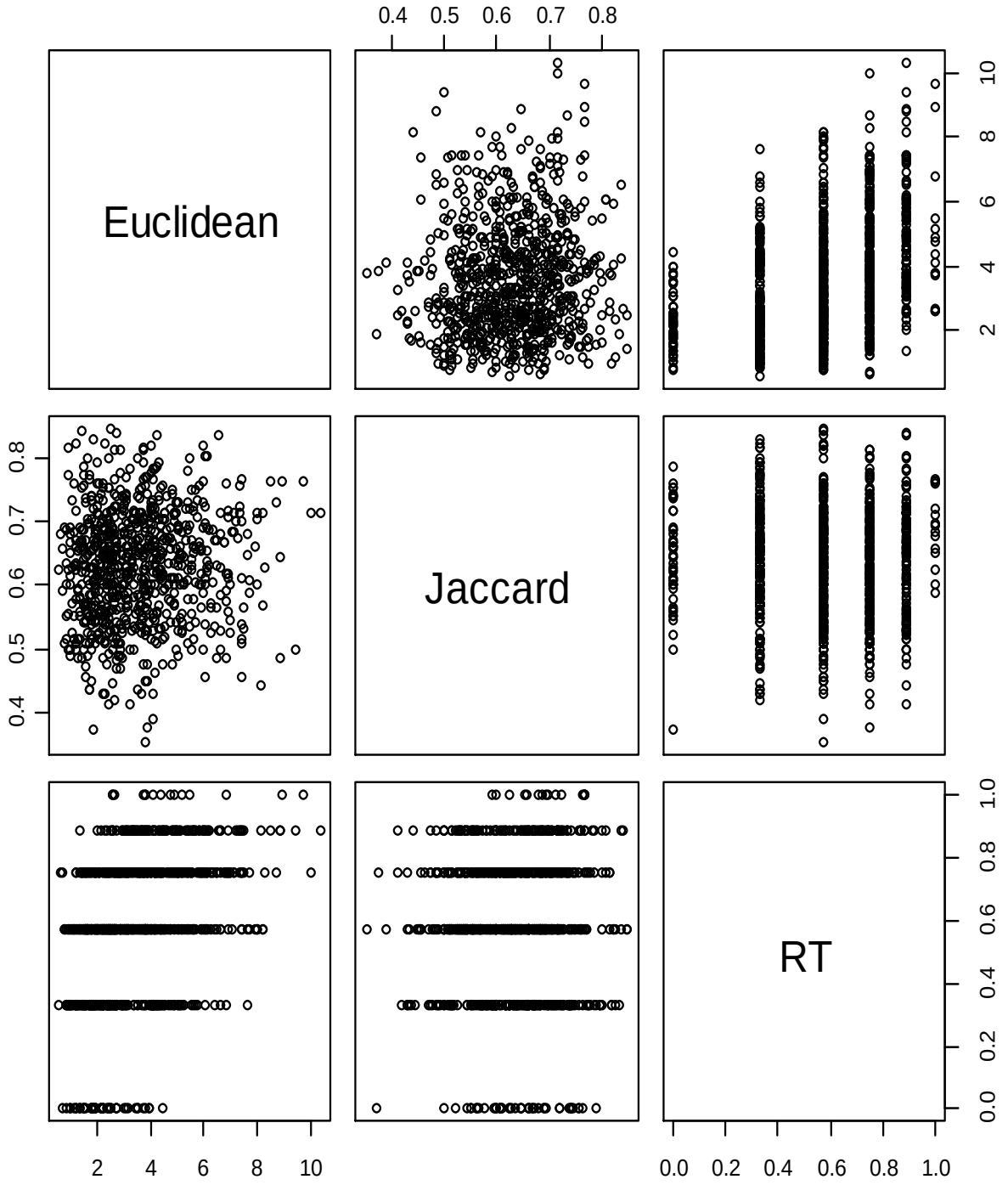
Appendix 11. Estimates of genetic distance based on SSR markers characters for all pair-wise comparisons of 40 red clover accessions

GE .No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	0.00																																							
2	0.7	0.00																																						
3	0.8	0.7	0.00																																					
4	0.7	0.8	0.7	0.00																																				
5	0.7	0.7	0.8	0.8	0.00																																			
6	0.8	0.8	0.8	0.8	0.7	0.00																																		
7	0.7	0.8	0.9	0.9	0.6	0.8	0.00																																	
8	0.6	0.8	0.8	0.8	0.9	0.8	0.7	0.0																																
9	0.7	0.8	0.7	0.9	0.8	0.8	0.8	0.8	0.00																															
10	0.7	0.7	0.7	0.8	0.7	0.8	0.8	0.8	0.7	0.00																														
11	0.7	0.7	0.7	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.00																													
12	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.6	0.00																												
13	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.7	0.7	0.7	0.00																											
14	0.6	0.7	0.7	0.7	0.8	0.8	0.7	0.8	0.8	0.8	0.6	0.7	0.7	0.00																										
15	0.7	0.8	0.7	0.8	0.8	0.7	0.7	0.7	0.6	0.8	0.7	0.7	0.8	0.8	0.00																									
16	0.7	0.7	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.8	0.6	0.7	0.8	0.6	0.6	0.00																								
17	0.7	0.7	0.7	0.8	0.8	0.7	0.8	0.8	0.7	0.8	0.5	0.7	0.6	0.5	0.7	0.6	0.00																							
18	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.7	0.7	0.6	0.7	0.7	0.7	0.00																						
19	0.8	0.8	0.8	0.9	0.6	0.7	0.7	0.9	0.8	0.6	0.7	0.7	0.7	0.8	0.8	0.8	0.8	0.9	0.00																					
20	0.5	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.6	0.6	0.7	0.6	0.6	0.6	0.7	0.7	0.8	0.00																				
21	0.6	0.7	0.7	0.7	0.8	0.7	0.7	0.8	0.6	0.8	0.6	0.7	0.8	0.7	0.6	0.8	0.7	0.7	0.8	0.6	0.00																			
22	0.7	0.7	0.7	0.8	0.7	0.8	0.6	0.7	0.6	0.8	0.6	0.6	0.8	0.8	0.7	0.5	0.7	0.7	0.7	0.6	0.7	0.00																		
23	0.8	0.8	0.7	0.8	0.8	0.7	0.7	0.7	0.7	0.8	0.8	0.8	0.7	0.8	0.6	0.7	0.7	0.8	0.9	0.7	0.7	0.7	0.00																	
24	0.7	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.8	0.8	0.7	0.7	0.8	0.7	0.6	0.6	0.7	0.7	0.8	0.6	0.7	0.7	0.7	0.00																
25	0.6	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.6	0.7	0.7	0.6	0.7	0.8	0.8	0.6	0.7	0.7	0.7	0.6	0.8	0.8	0.8	0.00															
26	0.7	0.7	0.8	0.8	0.7	0.7	0.8	0.8	0.8	0.7	0.6	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.8	0.00														
27	0.7	0.7	0.7	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.6	0.7	0.7	0.7	0.8	0.7	0.6	0.7	0.7	0.8	0.7	0.7	0.00													
28	0.6	0.8	0.8	0.8	0.7	0.8	0.7	0.8	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.6	0.7	0.8	0.6	0.7	0.7	0.00												
29	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.8	0.8	0.7	0.6	0.7	0.7	0.7	0.6	0.7	0.8	0.8	0.6	0.6	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.00											
30	0.7	0.7	0.7	0.7	0.6	0.8	0.7	0.8	0.8	0.7	0.6	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.7	0.5	0.7	0.6	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.00										
31	0.8	0.7	0.8	0.8	0.7	0.8	0.7	0.9	0.8	0.8	0.8	0.7	0.8	0.9	0.7	0.7	0.8	0.8	0.7	0.7	0.7	0.6	0.8	0.8	0.8	0.7	0.6	0.7	0.6	0.8	0.00									
32	0.8	0.8	0.7	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.8	0.8	0.7	0.6	0.7	0.00							
33	0.7	0.7	0.7	0.8	0.7	0.8	0.6	0.8	0.6	0.7	0.6	0.6	0.7	0.8	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.5	0.6	0.6	0.7	0.7	0.6	0.7	0.6	0.5	0.7	0.6	0.00							
34	0.8	0.7	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.7	0.8	0.8	0.7	0.00						
35	0.7	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.7	0.6	0.7	0.6	0.8	0.7	0.8	0.6	0.7	0.7	0.8	0.8	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.00					
36	0.8	0.7	0.8	0.9	0.7	0.7	0.7	0.8	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.8	0.7	0.8	0.7	0.7	0.6	0.7	0.6	0.6	0.6	0.6	0.8	0.8	0.00			
37	0.7	0.8	0.7	0.8	0.6	0.7	0.8	0.8	0.7	0.6	0.7	0.7	0.7	0.8	0.6	0.8	0.8	0.7	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.00				
38	0.7	0.8	0.8	0.9	0.8	0.8	0.6	0.7	0.7	0.8	0.7	0.6	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.6	0.7	0.7	0.7	0.6	0.7	0.7	0.8	0.7	0.6	0.7	0.8	0.8	0.6	0.7	0.7	0.6	0.6	0.00		
39	0.7	0.7	0.8	0.8	0.7	0.7	0.7	0.8	0.7	0.8	0.6	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.6	0.6	0.6	0.8	0.6	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.6	0.6	0.8	0.6	0.7	0.6	0.7	0.00
40	0.7	0.7	0.7	0.7	0.8	0.7	0.8	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.6	0.7	0.8	0.7	0.8	0.6	0.7	0.8	0.7	0.7	0.7	0.7	0.8	0.8	0.6	0.7	0.8	0.7	0.7	0.8	0.7	0.7	0.6	0.6	0.7	0.00

Appendix 12. Similarity matrix among 40 red clover accessions using the Rogers and Tanimoto's coefficient

GE .No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	0.0	0.5	0.7	1.0	0.5	0.8	0.7	0.8	0.3	0.8	0.5	0.8	1.0	0.7	0.7	0.8	0.3	0.8	0.7	0.7	0.8	0.8	0.8	0.7	0.8	0.8	0.7	0.3	0.5	0.5	0.7	0.7	0.8	0.7	0.5	0.8	0.8	0.5	0.7	0.8
2	0.5	0.0	0.7	0.7	0.5	0.5	0.3	0.5	0.3	0.8	0.5	0.5	0.8	0.3	0.3	0.5	0.7	0.5	0.3	0.5	0.7	0.5	0.5	0.3	0.5	0.5	0.7	0.3	0.0	0.5	0.3	0.7	0.5	0.7	0.5	0.5	0.5	0.5	0.8	0.5
3	0.7	0.7	0.0	0.7	0.7	0.3	0.5	0.3	0.8	1.0	0.7	0.3	0.5	0.5	0.5	0.3	0.5	0.3	0.5	0.7	0.3	0.3	0.3	0.7	0.3	0.3	0.5	0.8	0.7	0.7	0.5	0.5	0.7	0.7	0.5	0.5	0.5	0.8	0.7	0.8
4	1.0	0.7	0.7	0.0	0.7	0.5	0.5	0.5	0.8	0.5	0.8	0.5	0.5	0.7	0.5	0.5	0.8	0.5	0.5	0.7	0.7	0.5	0.5	0.7	0.5	0.5	0.7	0.8	0.7	0.7	0.5	0.7	0.5	0.8	0.8	0.5	0.5	0.8	0.8	0.7
5	0.5	0.5	0.7	0.7	0.0	0.5	0.3	0.5	0.3	0.7	0.5	0.5	0.8	0.7	0.3	0.5	0.3	0.5	0.3	0.3	0.7	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.5	0.0	0.3	0.3	0.5	1.0	0.8	0.5	0.5	0.5	0.5	0.8
6	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.0	0.3	0.5	0.3	0.0	0.0	0.5	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
7	0.7	0.3	0.5	0.5	0.3	0.3	0.0	0.3	0.5	0.8	0.7	0.3	0.7	0.5	0.0	0.3	0.5	0.3	0.0	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.5	0.5	0.3	0.3	0.0	0.5	0.3	0.8	0.7	0.3	0.3	0.7	0.7	0.7
8	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.0	0.3	0.5	0.3	0.0	0.0	0.5	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
9	0.3	0.3	0.8	0.8	0.3	0.7	0.5	0.7	0.0	0.7	0.3	0.7	1.0	0.5	0.5	0.7	0.5	0.7	0.5	0.5	0.8	0.7	0.7	0.5	0.7	0.7	0.5	0.0	0.3	0.3	0.5	0.5	0.7	0.8	0.7	0.7	0.7	0.3	0.7	0.7
10	0.8	0.8	1.0	0.5	0.7	0.8	0.8	0.8	0.7	0.0	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.0	0.8	0.8	0.7	0.8	0.8	0.7	0.7	0.8	0.7	0.8	0.7	0.8	1.0	1.0	0.8	0.8	0.7	0.8	0.8
11	0.5	0.5	0.7	0.8	0.5	0.5	0.7	0.5	0.3	0.7	0.0	0.5	0.8	0.3	0.7	0.5	0.7	0.5	0.7	0.7	0.7	0.5	0.5	0.7	0.5	0.5	0.3	0.3	0.5	0.5	0.7	0.3	0.8	0.8	0.7	0.7	0.7	0.3	0.7	0.7
12	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.0	0.3	0.5	0.3	0.0	0.0	0.5	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
13	1.0	0.8	0.5	0.5	0.8	0.5	0.7	0.5	1.0	0.8	0.8	0.5	0.0	0.7	0.7	0.5	0.8	0.5	0.7	0.8	0.3	0.5	0.5	0.8	0.5	0.5	0.7	1.0	0.8	0.8	0.7	0.7	0.7	0.8	0.8	0.7	0.7	1.0	0.7	0.8
14	1.0	0.8	0.5	0.5	0.8	0.5	0.7	0.5	1.0	0.8	0.8	0.5	0.7	0.0	0.7	0.5	0.8	0.5	0.7	0.8	0.3	0.5	0.5	0.8	0.5	0.5	0.7	1.0	0.8	0.8	0.7	0.7	0.7	0.8	0.8	0.7	0.7	1.0	0.7	0.8
15	0.7	0.3	0.5	0.7	0.7	0.3	0.5	0.3	0.5	0.8	0.3	0.3	0.7	0.5	0.0	0.3	0.8	0.3	0.5	0.7	0.5	0.3	0.3	0.5	0.3	0.3	0.5	0.5	0.3	0.7	0.5	0.5	0.7	0.7	0.5	0.5	0.5	0.5	0.8	0.5
16	0.7	0.3	0.5	0.5	0.3	0.3	0.0	0.3	0.5	0.8	0.7	0.3	0.7	0.5	0.3	0.0	0.5	0.3	0.0	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.5	0.5	0.3	0.3	0.0	0.5	0.3	0.8	0.7	0.3	0.3	0.7	0.7	0.7
17	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.7	0.0	0.0	0.3	0.5	0.3	0.0	0.0	0.5	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
18	0.3	0.7	0.5	0.8	0.3	0.7	0.5	0.7	0.5	0.8	0.7	0.7	0.8	0.8	0.5	0.7	0.7	0.0	0.5	0.5	0.7	0.7	0.7	0.7	0.7	0.7	0.5	0.5	0.7	0.3	0.5	0.5	0.7	0.8	0.7	0.7	0.7	0.7	0.5	1.0
19	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.3	0.0	0.5	0.3	0.0	0.0	0.5	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
20	0.7	0.3	0.5	0.5	0.3	0.3	0.0	0.3	0.5	0.8	0.7	0.3	0.7	0.5	0.0	0.3	0.5	0.3	0.3	0.0	0.5	0.3	0.3	0.3	0.3	0.3	0.5	0.5	0.3	0.3	0.0	0.5	0.3	0.8	0.7	0.3	0.3	0.7	0.7	0.7
21	0.7	0.5	0.7	0.7	0.3	0.5	0.3	0.5	0.5	0.8	0.7	0.5	0.8	0.7	0.3	0.5	0.5	0.5	0.3	0.7	0.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.5	0.5	1.0	0.8	0.5	0.5	0.7	0.7	0.8
22	0.8	0.7	0.3	0.7	0.7	0.3	0.5	0.3	0.8	1.0	0.7	0.3	0.3	0.5	0.5	0.3	0.7	0.3	0.5	0.7	0.3	0.0	0.3	0.7	0.3	0.3	0.5	0.8	0.7	0.7	0.5	0.5	0.7	0.8	0.7	0.5	0.5	0.8	0.5	0.8
23	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.0	0.3	0.5	0.3	0.0	0.0	0.5	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
24	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.0	0.3	0.5	0.3	0.0	0.5	0.0	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
25	0.7	0.3	0.7	0.7	0.5	0.5	0.3	0.5	0.5	0.7	0.7	0.5	0.8	0.5	0.3	0.5	0.7	0.5	0.3	0.5	0.7	0.5	0.5	0.5	0.0	0.5	0.7	0.5	0.3	0.5	0.3	0.7	0.5	0.8	0.7	0.5	0.5	0.7	0.8	0.7
26	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.0	0.3	0.5	0.3	0.0	0.0	0.5	0.0	0.0	0.3	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
27	0.8	0.5	0.3	0.5	0.5	0.0	0.3	0.0	0.7	0.8	0.5	0.0	0.5	0.3	0.3	0.0	0.7	0.0	0.3	0.5	0.3	0.0	0.0	0.5	0.0	0.3	0.0	0.7	0.5	0.5	0.3	0.3	0.5	0.8	0.7	0.3	0.3	0.7	0.7	0.7
28	0.7	0.7	0.5	0.7	0.3	0.3	0.5	0.3	0.5	0.7	0.3	0.3	0.7	0.5	0.5	0.3	0.5	0.3	0.5	0.5	0.5	0.3	0.3	0.7	0.3	0.3	0.5	0.0	0.7	0.3	0.5	0.0	0.7	1.0	0.8	0.5	0.5	0.5	0.5	0.8
29	0.3	0.3	0.8	0.8	0.3	0.7	0.5	0.7	0.0	0.7	0.3	0.7	1.0	0.5	0.5	0.7	0.5	0.7	0.5	0.5	0.8	0.7	0.7	0.5	0.7	0.7	0.5	0.3	0.0	0.3	0.5	0.5	0.7	0.8	0.7	0.7	0.7	0.3	0.7	0.7
30	0.5	0.0	0.7	0.7	0.5	0.5	0.3	0.5	0.3	0.8	0.5	0.5	0.8	0.3	0.3	0.5	0.7	0.5	0.3	0.5	0.7	0.5	0.5	0.3	0.5	0.5	0.7	0.3	0.3	0.0	0.3	0.7	0.5	0.7	0.5	0.5	0.5	0.5	0.8	0.5
31	0.5	0.5	0.7	0.7	0.0	0.5	0.3	0.5	0.3	0.7	0.5	0.5	0.8	0.7	0.3	0.5	0.3	0.5	0.3	0.3	0.7	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.5	0.3	0.0	0.3	0.5	1.0	0.8	0.5	0.5	0.5	0.5	0.8
32	0.7	0.3	0.5	0.5	0.3	0.3	0.0	0.3	0.5	0.8	0.7	0.3	0.7	0.5	0.0	0.3	0.5	0.3	0.0	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.5	0.5	0.3	0.3	0.3	0.0	0.3	0.8	0.7	0.3	0.3	0.7	0.7	0.7
33	0.7	0.7	0.5	0.7	0.3	0.3	0.5	0.3	0.5	0.7	0.3	0.3	0.7	0.5	0.5	0.3	0.5	0.3	0.5	0.5	0.5	0.3	0.3	0.7	0.3	0.3	0.0	0.5	0.7	0.3	0.5	07	0.0	1.0	0.8	0.5	0.5	0.5	0.5	0.8
34	0.8	0.5	0.7	0.5	0.5	0.5	0.3	0.5	0.7	0.8	0.8	0.5	0.7	0.7	0.3	0.5	0.7	0.5	0.3	0.5	0.7	0.5	0.5	0.5	0.5	0.5	0.7	0.7	0.5	0.5	0.3	0.7	0.7	0.0	0.8	0.5	0.5	0.8	0.8	0.5
35	0.7	0.7	0.7	0.8	1.0	0.8	0.8	0.8	0.8	1.0	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8	1.0	0.8	0.8	0.8	0.8	0.8	0.8	1.0	0.8	0.7	1.0	0.8	1.0	0.7	0.3	0.0	0.7	0.7	0.7	0.8	0.3
36	0.5	0.5	0.5	0.8	0.8	0.7	0.7	0.7	0.7	1.0	0.7	0.7	0.8	0.5	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.5	0.8	0.7	0.8	0.8	0.3	0.5	0.0	0.5	0.5	0.7	0.5
37	0.8	0.5	0.5	0.5	0.5	0.3	0.3	0.3	0.7	0.8	0.7	0.3	0.7	0.5	0.3	0.3	0.7	0.3	0.3	0.5	0.5	0.3	0.3	0.5	0.3	0.3	0.5	0.7	0.5	0.5	0.3	0.5	0.5	0.7	0.5	0.0	0.0	0.5	0.5	0.5
38	0.5	0.5	0.8	0.8	0.5	0.7	0.7	0.7	0.3	0.7	0.3	0.7	1.0	0.5	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.5	0.3	0.5	0.5	0.7	0.5	0.8	0.7	0.5	0.5	0.5	0.0	0.5	0.5
39	0.7	0.8	0.7	0.8	0.5	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.5	0.7	0.7	0.7	0.5	0.7	0.7	0.8	0.7	0.7	0.5	0.7	0.8	0.5	0.7	0.5	0.8	0.8	0.7	0.5	0.5	0.5	0.0	0.8
40	0.8	0.5	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.8	0.5	0.7	0.7	1.0	0.7	0.7	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.7	0.5	0.8	0.7	0.8	0.5	0.3	0.5	0.5	0.5	0.5	0.8	0.0

Appendix 13**. Associations among red clover quantitative traits by non-parametric Spearman correlation coefficient**



Прилог 1.

Изјава о ауторству

Потписани-а _____

број индекса _____

Изјављујем

да је докторска дисертација под насловом

_____

_____

- резултат сопственог истраживачког рада,
- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

Потпис докторанда

У Београду, _____

_____

Прилог 2.

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора _____

Број индекса _____

Студијски програм _____

Наслов рада _____

Ментор _____

Потписани/а _____

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис докторанда

У Београду, _____

_____

Прилог 3.

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

_____

_____

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство

2. Ауторство - некомерцијално

3. Ауторство – некомерцијално – без прераде

4. Ауторство – некомерцијално – делити под истим условима

5. Ауторство – без прераде

6. Ауторство – делити под истим условима

(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

Потпис докторанда

У Београду, _____

_____

1. Ауторство - Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце, чак и у комерцијалне сврхе. Ово је најслободнија од свих лиценци.

2. Ауторство – некомерцијално. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца не дозвољава комерцијалну употребу дела.

3. Ауторство - некомерцијално – без прераде. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, без промена, преобликовања или употребе дела у свом делу, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца не дозвољава комерцијалну употребу дела. У односу на све остале лиценце, овом лиценцом се ограничава највећи обим права коришћења дела.

4. Ауторство - некомерцијално – делити под истим условима. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца не дозвољава комерцијалну употребу дела и прерада.

5. Ауторство – без прераде. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, без промена, преобликовања или употребе дела у свом делу, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца дозвољава комерцијалну употребу дела.

6. Ауторство - делити под истим условима. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца дозвољава комерцијалну употребу дела и прерада. Слична је софтверским лиценцама, односно лиценцама отвореног кода.

BIOGRAPHY

Ramadan Salem A. Ahsyee was born 15.10.1972 in Zentani in Libya. In the same place he finished high school. Basic studies enrolled in 1990. from the Faculty of Veterinary Medicine, wild plants and Rural Development, University of Al-Fateh in Tripoli and graduated in 1994. Ramadan Salem A. Ahsyee, engineer of Agricultural Sciences, citizen of the Great Socialist People's Libyan Arab Jamahiriya.

Following his four-year full-time study, the candidate Ramadan Salem A. Ahsyee in 2000 / 2001th school year, passed the examination by the graduate program at the Faculty of Biology at Botanici. Master's thesis entitled "Effect of salt on the germination and growth of seedlings of ten varieties of palm trees," he defended at the University 7th April at the Department of Biology, Faculty of Natural Sciences, 2005. His master's degree is validated at the University of Belgrade 28 aprila 2010. And earned the right to register the thesis. Ramadan is an assistant at the Faculty of Science (Zentani), El-Gabal El-Gharby University, Libya. And he speaks English and Arabic.