ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Estimation of Genetic Diversity in commercial *Trifolium repens* reported from Pakistan using Biochemical Makers (SDS-PAGE)

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Manuscript Info

Manuscript History:

Received: 12 February 2014
Final Accepted: 22 March 2014
Published Online: April 2014

Key words:
Trifolium repens, genetic diversity, SDS-PAGE, crude seed protein.

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Abstract

Conservation of plant genetic resources has been widely exercised. The aim of the present study was to estimation the level of genetic diversity among 11 commercial *Trifolium repens* genotypes collected from different areas of Khyber Pakhtunkhwa, Pakistan through Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis. Based on proteomics homology; cluster analysis grouped 11 cultivars into two Lineages. Lineage-I gathered 7 cultivars, which was further divided into two clusters. Cluster-1 having 3 cultivars collected from Bannu[1], Bannu[3] and North Waziristan[2]. On the other hand cluster-2 sorted 4 cultivars collected from Bannu[2], Dir Upper[2] and Dir Upper[3]. Similarly, Linkage-ll genetically grouped 4 cultivars, which were collected from Sargoda, Mardan, Dir (L) and Dir (U)[1]. SDS-PAGE revealed a low level of genetic variation within commercial *Trifolium* cultivar collected from different agro-ecologically zones of Khyber Pakhtunkhwa, Pakistan.

Introduction

Trifolium repens (White-clover) belongs family Papillionaceae, Subfamily Papilionoideae and tribe Trifolieae (Ali, 1977; Williams 1987, Lewis et al., 2005). The repens is originated from the Mediterranean region of Europe and was spread through Europe and Western Asia with migrating animals before recorded history (George et al., 2006; Zhang et al., 2010). Chemically, the White-clover containing 22-28% protein, 2.7-3.3% fat, 9.4-11.9% ash, 6.6-7% lignin and 15.7-21.1% fiber content (Anonymous, 2005). Keeping in view the predominant out breeding nature and induced polyploidy, white clover populations containing high level of genetic polymorphism (Voisey et al., 1994). Documentation of plant genetic diversity is necessary to conserve genetic resources for plant improvement (Lane et al., 2000). Genetic characterization in different crop species has long been based on morphological traits; however morphological traits may be affected by environmental factors. Recently, biochemical and molecular techniques are emerging as a complementary strategy for characterization of the plant genome in conjunction with morphological traits aim to magnifying the level of genetic diversity for the crop improvement (Nisar et al., 2011). These techniques are reproducible and largely independent of environmental instability/factors (Ayad et al., 1995; Bretting and Widrlechner, 1995, Nisar et al., 2009). Increasingly, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) are widely used for estimation of taxonomic relationships among inter and intra related species and assessment of genetic diversity reported by different scientist (Ghafoor et al 2002, Karihaloo et al., 2002; Duran et al., 2005; Iqbal et al., 2005; Lioli et al., 2005; Yuzbasioglu et al., 2008). Interestingly, the present work is the first attempt to assess the level of genetic diversity in commercial cultivars of *Trifolium repens* through Biochemical Makers, collected from different agro-ecological zones of Pakistan.

Material and Method

Plant materials

A total of 11 different cultivars of *Trifolium repens* were collected from different areas of Khyber Pakhtunkhwa, which includes Dir (L) (Malakand division), Dir (U)[1], Dir (U)[2,] Dir (U)[3], North Waziristan[1] (FTA), North Waziristan[2], Bannu[1] (FTA), Bannu[2], Bannu[3], and Mardan; while single cultivar was from Sargoda (Punjab).

Protein extraction

To extract crude protein of 11 cultivars, 0.01g of seed powdered was added to 400µl protein extraction buffer (0.05 M Tris–HCl, 0.2% (w/v) SDS, 5 M Urea, 1% β-mercaptoethanol, 1 % (w/v) Bromophenol and maintain the pH 8). At that moment the mixture of seed powder and PEB in E-tube was centrifuge at 14000 rpm for 10 minutes (Sawhney and Randhir, 2007). The powdered debris was collected as pellet, while about 20µl supernatant containing the crude protein was run of 12% polyacrylamide gel through slab vertical gel electrophoresis machine. A constant current of 100 volt was used for 3 hours during electrophoresis. After complete run, the gels were stained with 0.5% coomassie brilliant blue (CBB) G-250 in acetic acid-methanol-water (3:22:25 volume ratio) for two hours and de-stained in acetic acid methanol- water (5:20:75 volume ratios) for overnight (Sadia et al., 2009).

Gel evaluation for data scoring was done on a light box. The experiment was repeated 3 times to check the reproducibility of the score-able protein bands. A band presence was coded (1), while the absence of bands scored as (0). The data was analyzed by using the software PC-ORD (McCune and Grace, 2005) because of the difficulties in the visual interpretation of SDS-PAGE of seed protein profiles.

Results

Information regarding genetic diversity is a key component for the development of novel and desirable traits containing *Trifolium* cultivar. The banding patterns of *Trifolium repens* are shown in figure 1. Maximum number of bands were observed in the cultivar collected from North Waziristan[1], Bannu[2], Dir (U)[2] and Dir (U)[3], while minimum numbers of bands were scored in the germplasm of Sargoda, Mardan, North Waziristan[2], Dir (L), Bannu[1], Bannu[3], and Dir (U)[1]. It was calculated that the polypeptide band number 1 and 2 showing genetic polymorphism while band number 3, 4 and 5 were monomorphic.

The data scored in the form of binary data matrix was subjected to statistical analysis. Two-ways cluster analysis (TWCA) using Ward's method sorted 11 germplasm into two linkage viz. Linkage-1 and linkage-2. Linkage-1 consists of single cluster (C-1) sorted three cultivars collected from Bannu[1], Bannu[3] and North Waziristan[2]. The cultivars of Bannu[2] and North Waziristan were inter-spread with the cultivars of Dir (U) [2] and Dir (U) [3] majorly grouped in C-2. While the cultivars collected from Sargoda (Punjab) were genetically homologous to the cultivars of Mardan, Dir (L) and Dir (U) [1] shown in C-3 (Fig 2).

The genetic association found through TWCA was reconfirmed through Scatter Plot (Fig 3) using Principal Component Analysis (PCA). The plot also identified the same genetic association among cultivars collected from different agro-ecological zones of Pakistan. These finding indicates that the cultivars of cluster-2 and cluster-3 were mixed; it might be the exchange of germplasm in these area. Similarly, C-1 purely sorted the germplasm of the Federal Territorial Area.

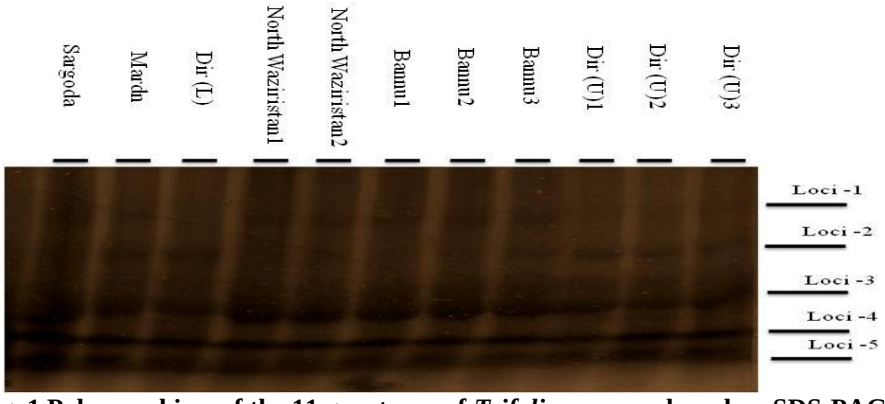

Fig. 1 Polymorphism of the 11 genotypes of *Trifolium repens* based on SDS-PAGE

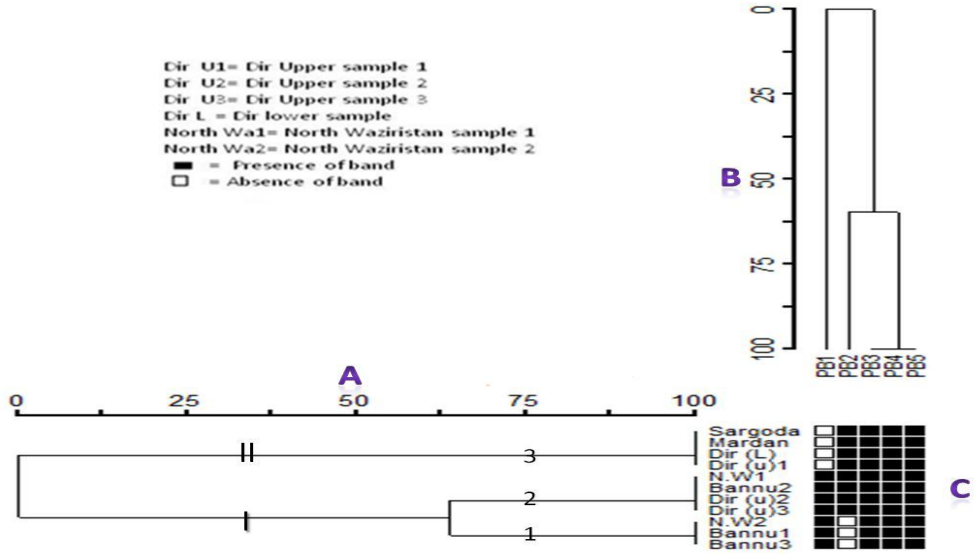

Fig. 2 Two way Cluster Analysis of Molecular Traits Matrix coding indication the presence and absence of protein bands using PCA: A; Cluster analysis of 11 among 11 cultivars of *Trifolium repens*. B; Genetic polymorphism based on protein polypeptide distributed in 11 cultivars of *Trifolium*. C; Zygomorph of 5 bands reported in indigenous *Trifolium* genotypes.

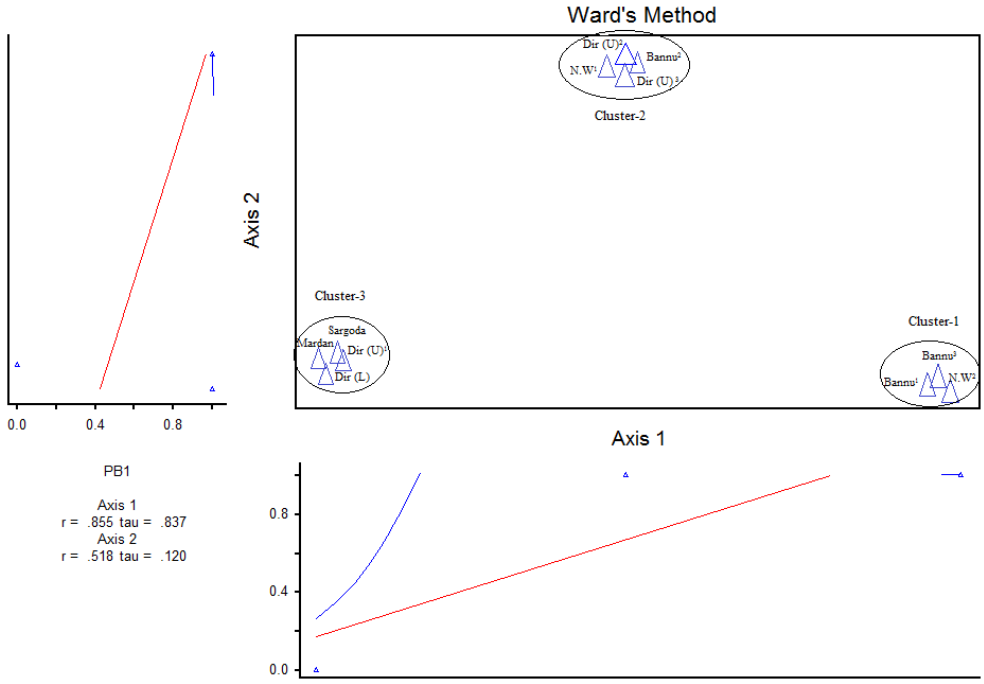

Fig. 3 A scattered plot grouped 11 genotypes of *Trifolium repens* into three cluster by using Principal Component Analysis (PCA).

DISCUSSION

Recently, several studies suggested that the application of numerical analysis, coupled with the utilization of protein patterns provides an effective approach for the investigation of taxonomic relationships among crop species (Karihaloo et al., 2002; Lioli et al., 2005; Yuzbasioglu et al., 2008). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of seed storage protein is a successful way of revealing genetic diversity and relationship among different taxa (Nisar et al., 2007). Genetic diversity in different plant species have been carried out by using electrophoretic patterns of total seed proteins as revealed by SDS-PAGE of seed storage protein (Ladizinsky and Hymowitz, 1979; Potokina et al., 2000; Ghafoor and Arshad, 2008; Ayten et al., 2009). In Leguminosae many studies have been carried out based on the electrophoresis of seed proteins (Hussein and George, 2002; Hussein et al., 2005). Some taxa of the genus *Trifolium* has been elaborated through seed storage protein using SDS-PAGE (Badr, 2000; Nikolic et al., 2010). Although in Pakistan the present study is a first documented attempt to find out the genetic diversity in *Trifolium repens* genotypes using SDS-PAGE.

The result clearly illustrated that eleven genotypes of Trifolium discriminated into three groups on the basis of their protein bands. However, it might be useful to distinguish diverse forms of Trifolium from one another. The findings indicated that for the discrimination of the *Trifolium repens* genotypes SDS-PAGE of seed proteins supplied additional banding patterns, however; the differentiations were not sufficient in distinguishing among the genotypes. The results were in partial agreement with the findings of Balkaya &Yanmaz (2002).

During present proteomic assays low level of diversity was observed for each locus in *Trifolium* genotypes. SDS-PAGE of seed protein profiles showed that each cluster had slight discriminative protein banding. The bands produce by SDS-PAGE among the 11 genotypes of *Trifolium repens* are varied among 1st locus (Dir L, Dir U^1, Sargoda and Mardan,) and 2^{nd} locus (Bannu[1], Bannu[3] and North Waziristan[2]).

According to the SDS-PAGE results, it can be suggested that genetic variation within *Trifolium* genotypes collected from Pakistan show narrow genetic base. Therefore, these primitive cultivated forms can be used in *Trifolium* breeding programmes to broaden the narrow genetic base of existing varieties as an assurance against unpredicted biotic and abiotic threats.

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