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CHARACTERIZATION OF PROTEIN IN DIFFERENT ACCESSIONS AND SPECIES OF *URGINEA*, HYACINTHACEAE

Banani Misra*[1], Hanumantha Rao[2], Shiva Kameshwari M.N.[3]

[1,2,3]Department of Botany, Bangalore University, Bangalore-560056
Corresponding Author Email - bananimisra561@gmail.com

Abstract: **The current study presents investigations on the characteristic of protein in different accessions of *Urginea indica/ Drimia indica* belonging to family Hyacinthaceae is a large polytypic genus of bulbous herbs. Different accessions of *Urginea indica* were collected from different regions of India like Ramanagar, Rangantitu, Gopalaswamibetta, sitampoondi, kerala and used for study. We have conducted our study using bulb part of the plant. Bradford method was used for estimation of protein. Highest protein was found in Rangantitu accession of *Urginea indica* around 0.88µg/ml. In Gopalaswamibetta accession the protein concentration was found to be 0.83µg/ml, Sitampoondi accession 0.75µg/ml, Ramanagar it was 0.71µg/ml and lowest protein concentration was seen in Kerala accession nearly 0.56 µg/ml. Native 12% poly acrylamide gel electrophoresis was carried out for characterization of protein in different accessions of *Urginea indica.* To see the diversification among species, three species of *Urginea*; *Urginea indica, Urgnea wightii* and *Urginea polyphylla* were taken into consideration for 12% native and SDS-PAGE.**

Keywords: **Protein, Native and SDS-PAGE, *Urginea.***

1. INTRODUCTION

Proteins are important compounds as these are associated with several aspects of our life. These are included in the form of detergent, food, cosmetics and other compounds for our daily life requirements. In biochemistry, proteins can be easily extracted from plant parts and analyzed with SDS-PAGE techniques. The proteins in the SDS-PAGE generates specific banding patterns with respect to specific plants, this respective banding patterns generates higher level of genetic polymorphism based on difference in the protein intensity among the genotypes and species. This method of protein separation in SDS-PAGE techniques is commonly used to explore the genetic diversity and to classify plant varieties; this method is also called as protein marker method. Morphological, biochemical, and molecular markers can be used to study genetic diversity. (Gonçalves et al., 2008) The protein marker method is very useful in study plants with respect to its environmental habitat because even ecological, ecosystem and climatic factors also influences variations of protein among different accessions and species of plants.

The biodiversity of organisms has great impact on the functioning of the natural ecosystem services and ecological processes. ecosystem benefits in different ways those are involved in the production of renewable resources such as food, wood, increased carbon sequestration, photosynthesis, recycling of nutrients, air and water purification, pollination, prevention of soil erosion etc, and regulating services are those that minimize environmental changes such as climatic change, controlling pest/diseases.(Pathak and Abido, 2014). Genetic differences among tree species, their hybrids and within tree species are known to influence associated ecological communities and ecosystem (Zytynska et al., 2011).

The major advantage of using protein as a marker to investigate the diversity of plants are absence of epistasis and pleiotropic effects, easy to use and comparatively cost effective and powerful method to explore plant genome polymorphism (E.A. Haliem 2013).

In addition, the isoenzymes are multiple forms of a single enzyme that present within a single species of plant (Binu Thomas 2012) are also having importance in plants diversity analysis. The isoenzyme profiling using Native-PAGE is an important biochemical technique to observe specific enzyme activity on gel by using respective substrates reagents. The combination of Isoenzymes and total protein banding patterns has been used to identify species, cultivars, inbred lines and aneuploids in the plants (Binu Thomas 2012). Basically, the standardization of Native-PAGE and SDS-PAGE method for any plants are very important intial step to perform any polymorphic study design. In general, use of polyacrylamide gel electrophoresis is a relatively simple, rapid, and highly sensitive tool to study the properties of proteins.

Native PAGE is one of the most powerful techniques for studying the composition and structure of native proteins, since both the conformation and biological activity of proteins remain intact during this technique (Davis, B. J 1964). But sometimes it is difficult to find standard proteins that resemble the shape, partial specific volume and degree of hydration as the native protein under investigation (Hames, B. D. 1981). Among different variants of this technique, sodium dodecyl sulfate (SDS)-polyacrylamide discontinuous gel electrophoresis, originally described by Laemmle, is the most commonly used system in which proteins are fractionated strictly by their size. This procedure denatures proteins and hence cannot be used to analyses native proteins and proteins whose biological activity needs to be retained for subsequent functional testing. On such occasions, it is necessary to use a non-denaturing system. Thus several factors may change protein structure like Ph, temperature and presence of other molecules like urea and mercaptoethanol, can lead to unfolding. Solutions containing high concentrations of alcohols can also denature proteins, as these alcohols compete with the hydrogen bonding between different parts of the protein.

Urginea indica is one of the important members of family Hyacnthaceae presents promising future prospects in view of their multiple uses. All parts of the plants like bulbs, roots and leaves are reported to possess medicinal properties. It is used as an expectorant, cardiac stimulant, asthma, dropsy, edema, gout, anti-inflammatory, male sterility, dog bite, allergies rheumatism (Khare, 2004). Two glycoproteins isolated from the bulbs have shown fungistatic effect and anti-cancerous properties (Deepak et al. 2003). Karyological characterization revealed the presence of diploid, triploid, tetraploid, pentaploid, hexaploidy and aneuploid accessions of *Urginea indica* (Shiva Kameshwari et al. 2010).

The present study revealed the protein profile in different ploidy level of *Urginea indica* collected from five different regions of India. Also, we have used both SDS and Native Polyacrylamide gel electrophoresis to identify total proteins and functional proteins present in three species in *Urginea* such as *Urginea indica, Urginea wightii and Urginea polyphylla.*

2. MATERIALS AND METHODS

Collection of Plant

Five accessions of *Urginea indica* collected from different regions from India like Ramanagar, Rangantitu, Gopalaswamibetta, sitampoondi, kerala.

Three species of *Urginea* collected from various localities in India such as Karwar (*Urginea indica*), Yediyur (*Urginea wightii*) and Poona Highway (*Urginea Polyphylla*).

Preparation of the extract and Protein Estimation

2 gram of plant material of each accession was taken and homogenized using 1M Phosphate buffer (pH-6.8). The homogenate was centrifuged at 5000rpm for 10 minutes. The supernatant was used for the quantification of proteins**.** Bradford method of protein estimation was carried out using Coomassie brilliant blue G-250 staining reagent and Bovine serum Albumin as standard. Bradford method of protein estimation depends on the binding of the dye Coomassie blue G-250 to protein. Coomassie Brilliant Blue G-250 (100mg) was dissolved in 50 ml of 95% ethanol. To this solution 100 ml 85% of Phosphoric acid was added and filtered using whattmann's filter paper to remove the debries. Then it is stored for further use. Bovine serum Albumin standard solution of 100µg/ml was prepared. Dilutions were taken and using 5 ml of dye reagent absorbance were taken at 595nm. The intensities of blue-green color change were observed.

Polyacrylamide gel electrophoresis (PAGE)

Poly acrylamide gel is a standard and important tool in laboratory to identify and analyze protein of interest. It may also be important to correlate a biochemical activity of a protein with a particular band on the gel. (Harry towbin et al. 1979). Native poly acrylamide gel electrophoresis was carried out using bulb extracts of the plant following the method of Laemmle et al 1970.

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Native polyacrylamide gel electrophoresis

Native polyacrylamide gel electrophoresis is used for separation of active proteins, enzymes and isoenzymes. Usually the protein samples prepared without adding SDS in buffer and in both separating and stalking gels, this method maintain protein structures and biological activity so Native-PAGE is important techniques in study of isoenzymes diversity across plant species. So 12 % separating gel and 4% stacking gel without SDS was prepared for protein separation.

Sample Preparation and Gel Running

Prior to adding the sample buffer to protein, kept all the samples at -4^{0}C. 100µl protein samples dissolved in sample buffer were loaded carefully to respective lane. Then the gel was applied to 200V for running. Gel was allowed to run till 4 hours and removed the gel from the cassette when dye front reached till the end of the gel apparatus and touches the running buffer.

Staining solution (500ml) was prepared using Methanol, Acetic acid, distill water, and comassie Brilliant Blue. The gel was removed from the cassette and kept the gel in the staining solution overnight. Washed the gel 2-3 times in distill water and destained the solution by several changes of de-staining solution of Methanol, Acetic acid and water in the presence of a sponge.

12 % Native and SDS-PAGE

Three different species *of Urginea, Urginea indica (U.indica), Urginea wightii (U.wightii), and Urginea polyphylla (U.polyphylla)* were taken to study total protein and functional proteins using 12% and 15% native and SDS PAGE.

Sample preparation

100µl of samples loaded onto the wells of each lane maintaining the protein concentration in all the three species for both Native and SDS PAGE.

Cluster analysis

The DendroUPGMA online program was used for developing Dendrogram and UPGMA clustering to indicate the genetic relationships among different accessions and species of *Urginea*.

3. RESULTS AND DISCUSSION

Bradford method was used for estimation of protein. Highest protein was found in Rangantitu accession of *Urginea indica* around 0.88µg/ml. In Gopalaswamibetta accession the protein concentration was found to be 0.83µg/ml, Sitampoondi accession 0.75µg/ml, Ramanagar it was 0.71µg/ml and lowest protein concentration was seen in Kerala accession nearly 0.56 µg/ml. Standard graph of bovine serum albumin has been shown in Fig-1.

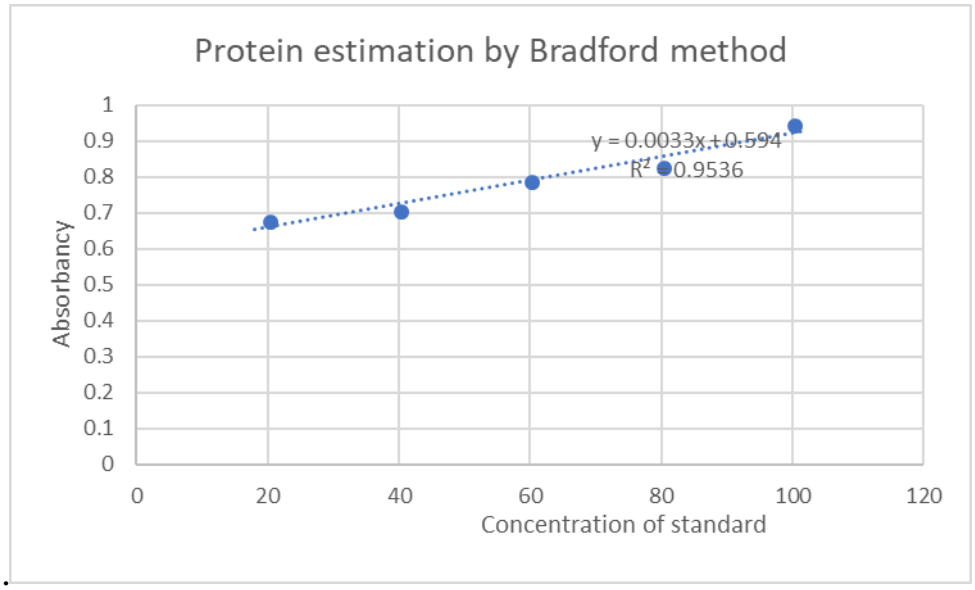

Fig 1: Protein estimation by Bradford method

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Native PAGE was carried out to notice the functional bands and also different molecular weight of Protein were observed in each accession. In Ramanagar accession three Protein bands 81kDa, 68. kDa, 60 kDa, in Rangantitu accession Four bands 81 kDa, 68 Kda, 54.5 Kda and 47kda were seen whereas in Gopalaswamybetta accession four bands with 97.4 kDa, 68 Kda, 66 kda, 54.5 kda and Sitampoondi accession Three bands with 68 kDa, 66 kDa and 54.5 kDa were seen. While in Kerala accession Four protein bands with approximately 158.2 kDa, 68 kDa, 66 kDa, 54.5 kDa protein bands were seen.

Thus 68 kDa proteins are common in all five accessions of *U.indica* and 54.5 kDa Protein was found in all four accessions except in Ramanagar. While 66kDa and 54.5 kDa was commonly found in gopalaswamybetta, Sitampoondi and Kerala accession. Unique single band was noticed in Gopalaswamibetta (97.4 kDa), Kerala (158.2 kDa), Ramanagar (47 kDa) and Rangantittu accession(47kDa).81 Kda and 68 kda is found in accession Ramanagar and Rangantitu. Thus, Ramanagaram and Rangantitu shares almost similar protein bands (Fig-2). Variation among different accession were found. This may be attributed due to the different ploidy level among different accession. It can also be due to climatic and Edaphic factor.

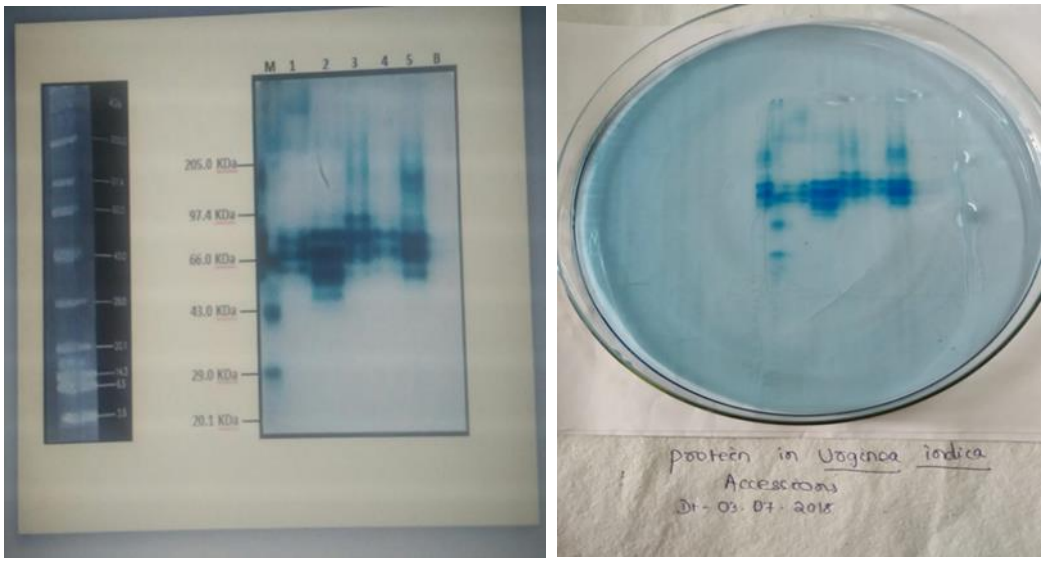

Fig 2: Proteins in Five different accessions of *Urginea indica*

UPGMA clustering dendrogram based on Native -PAGE analysis

The upgma method has been chosen for genetic diversity analysis. 5 rows with 8 variables in each row have been analyzed. The Jaccard coefficient has been used to compare between sets of variables **(Fig-3).**

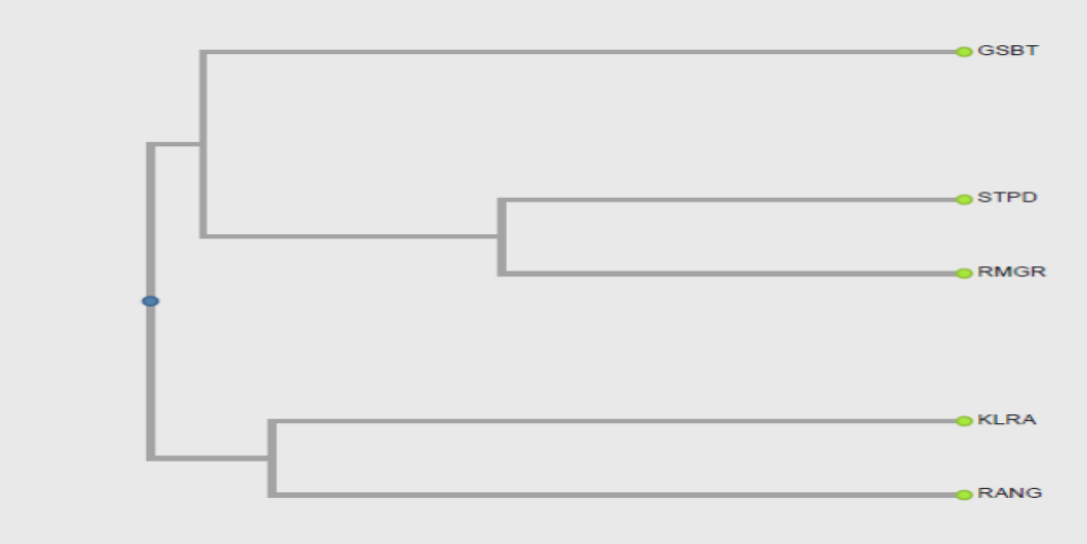

Fig 3: Dendrogram representing genetic relationship between different accession of *Urginea*

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The Cophenetic Correlation Coefficient (CP) value was found to be 0.79%.

This variations observed in different accessions of *U.indica* paved the way to explore variations at species level. Hence 12% native and SDS-PAGE was analyzed in three different species of *Urginea; Urginea indica, Urginea wightii and Urginea polyphylla* and following bands were observed.

In 12 % SDS PAGE- Molecular weight of protein observed in bulb1(*Urginea indica*)- 60 kDa, 50 kDa and 40 kDa bulb2(*Urginea wightii*)- 50 kDa,30 kDa,40kDa and 20 kDa and bulb3(*Urginea polyphylla*)- 60 kDa,50 kDa,30kDa and 40 kDa respectively**.**

whereas in 12% Native PAGE-Molecular weight of bulb1(*Urginea indica*)- 70, 50 and 29 KDa, bulb2(*Urginea wightii*)- 70 and 50 KDa and in (*Urginea polyphylla*)- 70, 50, 35 and 25kDa protein band respectively which has been portrayed in fig-4 and 5.

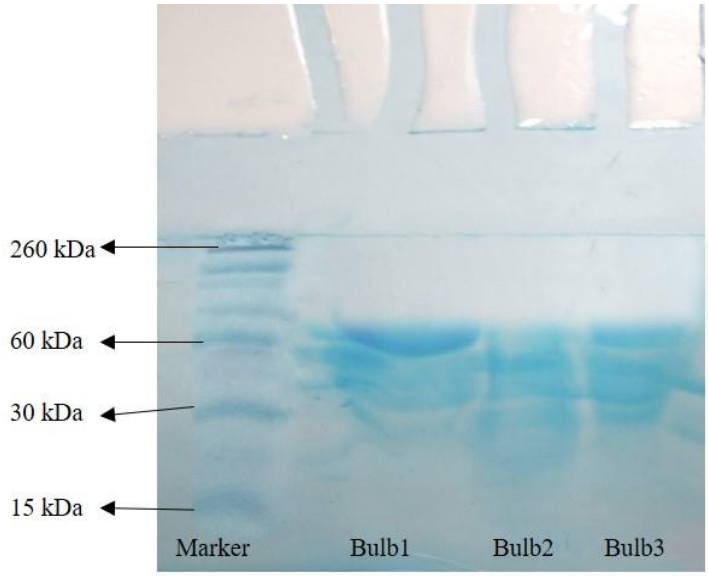

Figure 4: Protein profiling of 3 species of *Urginea* bulbs on 12% SDS-PAGE gel

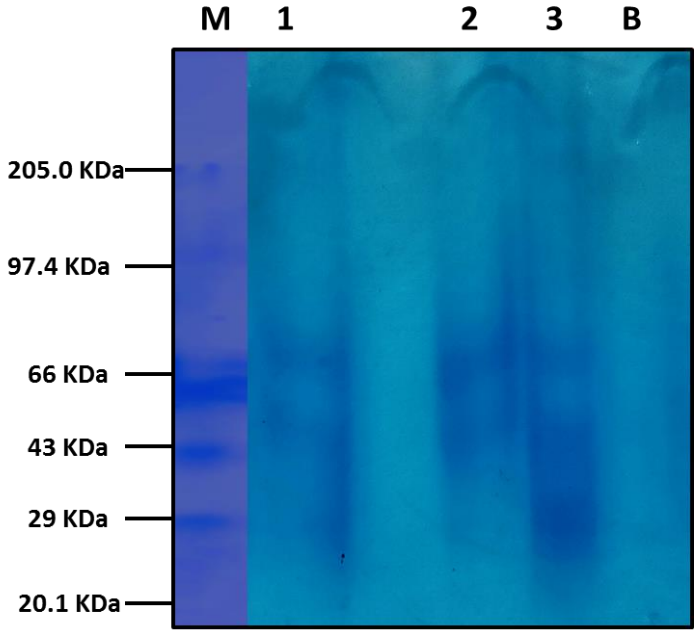

Figure 5: Protein profiling of 3 species of Urginea bulbs on 12% NATIVE-PAGE gel

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12 % SDS gel showed three protein band of 60 kDa, 50 kDa and 40 kDa is common in all three species of *Urginea* whereas unique band of 50 kDa is present in *Urginea wightii* and 30 kDa is present in *Urginea polyphylla.* whereas in 12 % native gel protein band of 70 and 50 kDa is common in all the three species but unique band of 29 KDa is present in *U.Indica* and unique band of 35 kDa and 25 kDa is present in *Urginea polyphylla*.

UPGMA clustering dendrogram based on SDS -PAGE and Native-PAGE analysis

The upgma method has been chosen.3 rows with 4 variables in each row have been analyzed. The Jaccard coefficient has been used to compare between sets of variables.

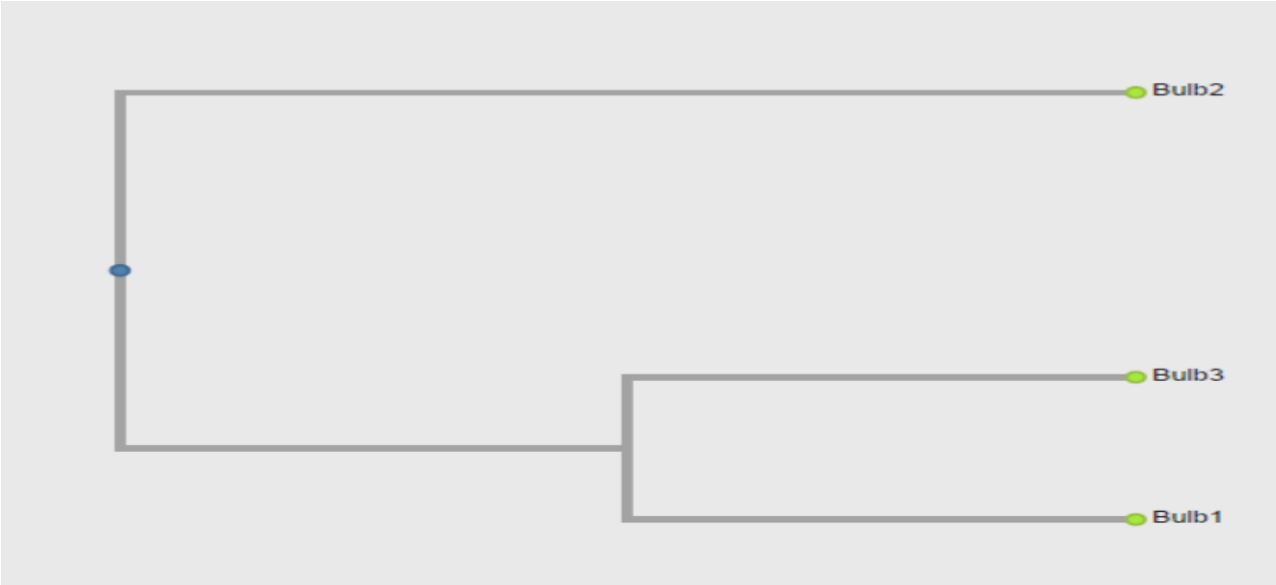

Fig 6: Dendrogram representing genetic relationship between different species of *Urginea based on 12% SDS-PAGE analysis*

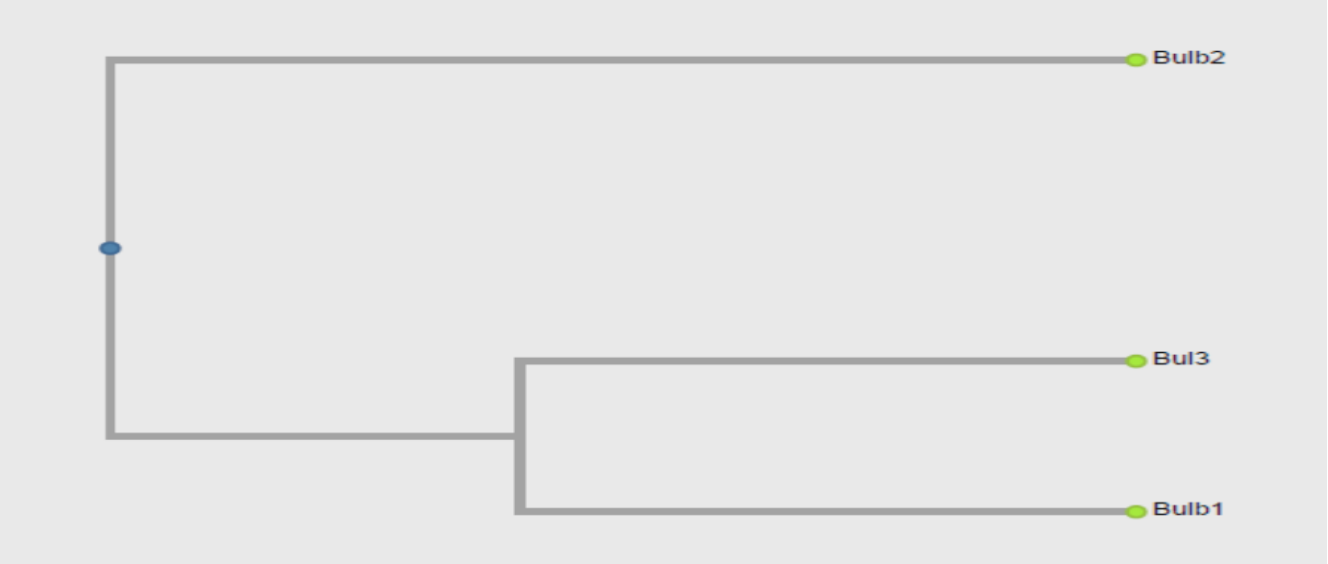

Fig 7: Dendrogram representing genetic relationship between different species of *Urginea based on 12% Native-PAGE analysis*

The Cophenetic Correlation Coefficient (CP) value is found to be 0.82% for 12 % SDS-PAGE gel whereas in 12% native-PAGE the CP value was approximately 0.75%.

Anda Linina et al. reported wheat cultivars with differences in their genetic as well as weather conditions significantly influence protein formation in grains**.** Lectin from *Cicer arietinum* showed one band with molecular weight of approx. 22 kDa as confirmed by SDS-PAGE (Balaji P, 2012). Protein profiles of tissue-cultured and farm cultivated *Gracilaria changii* were produced by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Lin WeiJonga et al.

2015). There were no remarkable variations were observed whereas in *Urginea* species there were noticed variation among accession also and different species. Microscale techniques like Clear native electrophoresis and blue native electrophoresis are studied to isolate the membrane protein complex (Ilka Wittig et al. 2007). Leo G. J Nijtmans, 2002 studied blue native-PAGE to isolate membrane and other protein complexes. G protein-coupled receptors (GPCRs). Phosphoprotein Quercetin has been detected by SDS-PAGE (Xi Wang et al. 2014). They have used a fluorescence based staining method for phosphoprotein analysis. Andrew et al. 2015 introduced blue native PAGE and native SDS-PAGE to show separation of Protein with retention of native properties. Characterization of proteins in the storage organs of *Curcuma alismatifolia* Gagnep. has been reported by Chamaiporn anuwong et al. (2014). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles showed that peptides of 10.6 kDa and 12.0 kDa found in Rhizome and are different from the protein present in storage root. Two novel type I ribosome-inactivating proteins (RIPs) were reported in the storage roots of *Mirabilis expansa*, an underutilized Andean root crop and their potential role in root-pathogen interactions (Vivanco et al.1999)**.** In *Urginea* species Bulb is the most essential part which showed different types of Banding pattern.

4. CONCLUSION

The findings presented in this study contribute to address several biological questions concerning the evolution of the relationship between genomes of different species. Indeed, role of gene functions and ploidy level can accelerate the diversification. Thus, genotypic and Ecological influences have played a major role in showing variations in protein in different accessions and species level of *Urginea.* Further, standardization of Native gel in this work for urginea proteins will research material for further analyzing protein-protein interaction in 2-D electrophoresis.

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