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Biochemical and Molecular Characterization of Castor Bean (*Ricinus Communis* L.) Collected from Different Climatic Zones of Pakistan

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Abstract: Castor oil plant (*Ricinus communis* L.) having imperative medicinal and industrial significances. In the present study, we observed the biochemical and molecular characterization of castor bean (*Ricinus communis* L.) collected from different climatic zones of Pakistan (Lahore, Peshawar, Rawalpindi, Dera Ismail Khan, Swat and Kohat). The protein banding pattern of all 6 accessions was found same and no specific variation was noticed among the proteins of high molecular weight. However, small amount of variation was found in the number and intensity of low molecular weight. RAPD analysis of four primers (OPA-01, OPA-03, OPA-09 and OPA-10) bares no variation in the genomic DNA banding patterns. Variations were found in the photosynthetic pigments attributes of the studied genotypes with high Chlorophyll *a*, *b* and total Carotenoids contents in plants of Kohat zone while lower level was found in plants of Swat zones. The present study revealed no variation among the genotypes by SDS-PAGE and RAPD. However it is useful to distinguish different form of castor with special reference to photosynthetic pigments grown in diverse climatic zone.

Key words: Castor oil plant, SDS-PAGE, RAPD, Photosynthetic Pigments, Pakistan

Introduction

Castor (*Ricinus communis* L.) belongs to the family Euphorbiaceae a none edible but used for with important medicinal and industrial applications. It is an indigenous plant species of Asia and Africa and habitually plentiful beside watercourses, floodplains, semiarid tropic and sub tropic sandy areas and stream banks etc. (DAFF, 2014). Costar

is generally cultivated for its high composition of oil containing (40-60%) which is mostly utilizes in industries and also as a source of high nitrogen fertilizer and in medication as a purgative, anti-flushing, and laxative (Allan et al., 2007). It is also possible to use as a biological bat due to it very noxious protein (Ilavarasan et al., 2006). It can be as a conventional medicine

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to convey chemotherapy cocktails to tumors and possesses high antibacterial activity against common human pathogenic micro flora (Malook et al., 2013; Fetrow and Avila, 1999). Different climatic features such as soil characteristics, rain fall, latitude and altitude, nitration, precipitation, temperature and intensity of light influencing the medicinal plants and their bioactive compounds (Omidbaigi, 2009; Endrias, 2006). As physico-chemical and molecular study of medicinal plant is imperative for its characterization and eminence of the bioactive compounds for further genetic diversity valuations of different crop species and is also important in term of its improvement and their establishment in an environment. In this investigation, we investigate biochemical and molecular characterization of castor bean (*Ricinus communis* L.) collected from different climatic zones of Pakistan (Lahore, Peshawar, Rawalpindi, Dera Ismail Khan, Swat and Kohat).

Materials and Methods

Plants Collection and Identification

Plants of *Ricinus communis* L. were collected from different environmental zones of Pakistan as Lahore, Peshawar, Rawalpindi, Dera Ismail Khan, Swat and Kohat regions (Table 1), and were identified according to the standard procedure of I.C.B.N (International Committee for Botanical nomenclature). Seeds and Leaves were separated from the plants, washed with distilled water, dried under shade and processed for antimicrobial activity (Malook et al., 2013). Some were stored for the analysis of photosynthetic pigments i.e. Chl "a", Chl "b" and total carotenoid contents, while some fresh leaves were stored at ultra low temperature (-80°C) for RAPD and SDS-PAGE analysis. The seeds were

completely grinded to fine powder with the help of electrical grander for determination of Seed storage proteins (SSP).

Table 1: Details of the castor oil plant collected from different climatic zones of Pakistan

S. No	Varieties	Locations	Geographical Locations
01	L1	Swat	35° north latitude and 72° and 30° east longitude
02	L2	Peshawar	34° 0' 28" North, 71° 34' 24" East
03	L3	Kohat	33°35'12" North, 71°26'31" East
04	L4	Rawalpindi	33° 36' 0" North, 73° 4' 0" East
05	L5	Lahore	31° 32' 59" North, 74° 20' 37" East
06	L6	Dera Ismail Khan	31° 49' 58" North, 70° 54' 9" East

Chlorophyll a, b and total Carotenoids Determination

Photosynthetic pigments were determined following the method of Lichtenthaler and Wellburn (1985) with slight modifications as suggested by Jamil et al. (2013). Equal amount of dry plant leaves (25 mg) and magnesium oxide were taken in 15ml plastics tube and placed for 1 hour under room temperature. In each tube, 5 ml HPLC grade methanol was added to the mixture of grinded leaves and magnesium oxide and completely homogenized the mixture with electrical homogenizer and placed on shaker for 2 hrs at room temperature. After complete homogenization, turbid mixture of pigments was transfer into centrifuge tubes. Centrifugation was done for 5 min at 4000 x g in an explosion-proof centrifuge at room temperature. After this the supernatant was transferred into cuvettes and the absorbance was noted at three wavelengths as 666 nm,

653 nm and 470 nm, using methanol as blank in a spectrophotometer. The Chlorophyll *a*, *b* and total Carotenoids were determined using the following formulas:

$$Ca = 15.65 A_{666} - 7.340 A_{653}$$

$$nC_b = 27.05 A_{653} - 11.21 A_{666}$$

$$C_{x+c} = 1000 A_{470} - 2.860 Ca - 129.2 C_b / 245$$

Characterization of seed storage proteins (SSP) and leaf (cell membrane embedded) proteins by SDS-Page

Extraction of seed storage proteins

For the extraction of seed storage proteins, the seeds were uncoated and crushed into fine powder. About 0.01 g of each sample was homogenized in 600 μ L protein extraction buffer with composition of 0.125 M Tris-HCL (pH 6.8), 8 M Urea, 4% SDS, 20% Glycerin and 5% 2-mercaptoethanol (Hong et al., 2001), kept overnight at 25 °C and then in oven at 40 °C for 30 minute and centrifuged at 13000 rpm for 10 minutes. The supernatant was removed and stored at 4 °C for further analysis.

Extraction of Cell membrane embedded proteins

For this purpose, leaves were crushed in liquid nitrogen with the help of mortar and pestle. Sample of 15 mg each was added in 400 μ L protein extraction buffer (PEB) with composition of Tris- HCL 0.5 M (pH 8), 2.5% SDS, 10% Glycerol, and 5% 2-mercaptoethanol. The solution was kept overnight at 40 °C and then centrifuged (10000 rpm) for 13 minutes at 25 °C. The supernatant was transferred into new 2 ml tubes and stored at 4 °C for further studies. Gel electrophoresis was done at 80 V for 2 and half hours according to the method of Sambrook et al. (2006). For staining Coomassive Brilliant Blue (CBB) for 40 minutes was used and for destaining 20% methanol and 5% acetic acid was used.

Extraction of Total Genomic DNA

Total Genomic DNA was extracted from the fresh leaves by the modified CTAB (Cetyltrimethylethyl Ammonium Bromide) method as describe by Dhakshanamoorthy et al. (2009), for obtaining good quality DNA, PVP (polyvinyl- pyrrolidine) was added. The quality and purity of DNA was calculated by spectrophotometer using A260/A280 absorbance ratio. DNA was further diluted with de ionized water to 50 ng/ μ L for RAPD amplification.

RAPD PCR amplification

DNA amplification was performed based on Williams's methodology (Williams et al., 1990). Polymerase chain reaction was performed in a PCR mixture of 25 μ L having composition (10X buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1U Taq DNA polymerase, 0.2 picomole primers, 50 ng of template DNA and PCR water). Initial denaturation of the DNA at 94 °C for 5 min, annealing at 37 °C for 1 minute and extension at 72 °C for 1.5 minutes and final extension at 72 °C for 10 minutes (Lal, 2011).

RAPD primers

A total of four RAPD primers (Operon Technologies (OT), Alameda, CA, USA) were used. Sequences of OPA (1, 3, 9 and 10) are shown in Table 2.

Table 2: List of the OPA Primers used during RAPD analysis

S.No	Primers	Primers Sequences
01	OPA-1	5'-CAGGCCCTTC-3'
02	OPA-3	5'-AGTCAGCCAC-3'
03	OPA-9	5'-GGGTAACGCC-3'
04	OPA-10	5'-GTGATCGCAG-3'

Results

Our result showed that three major bands of 30, 35 and 18 KDa related to seed storage proteins (SSP). While in leaves low intensity poly peptide band were observed in all accessions collected from different climatic zones of Pakistan. Low variations were found in all accessions collected from different climatic zones (Fig. 1). The Ricin, proteins (highly toxic) having 60 – 65 KDa molecular weights was in unreduced form while when treated with mercaptoethanol, protein was split in to two subunit of 30 – 32 KDa polypeptides. RAPD analysis showed no polymorphism which means no distinct variation was found between the studied accessions collected from different climatic zones of Pakistan (Fig. 2).

No variations were observed in their genomic DNA of all accessions by using

four primers (OPA- 1, 3, 9 and 10). Photosynthesis is determined by various environmental factors such as temperature, amount of carbon dioxide, water and minerals content in soil. In our results high chlorophyll contents were noted in castor oil plants collected from Kohat region i.e. 9.35, 56.40 and 2097.36 mg/g for Chl *a*, Chl *b* and total carotenoids, respectively while lower amount was found in the plant samples collected from Swat regions (2.39, 9.39 and 1721.90 mg/g) , respectively (Table 3). Similarly, plants collected from Dera Ismail Khan also showed lower level of photosynthetic pigments.

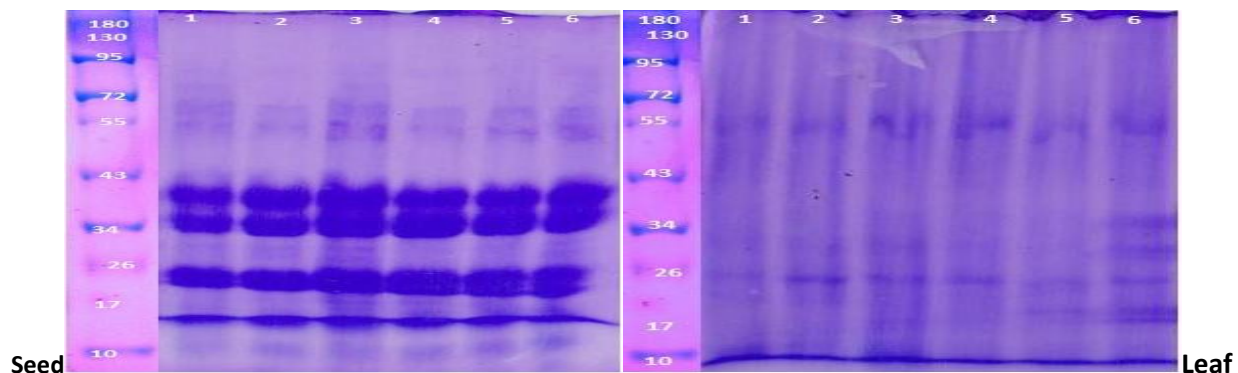


Fig 1. SDS-PAGE of seed storage proteins and leaf entrenched Proteins. Lane 1 (Swat), Lane 2 (Peshwar), Lane 3 (Kohat), lane 4 (Rawalpindi), Lane 5 (Lahore) and Lane 6 (Dera Ismail Khan).

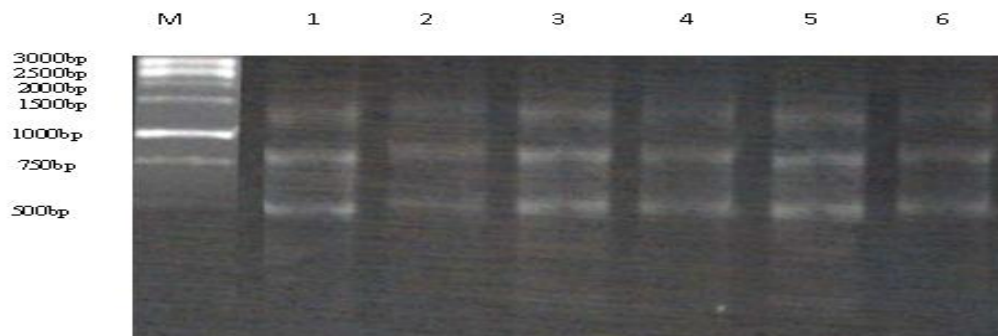


Fig. 2. RAPD pattern generated using primer OPA-10, Lane M (1kb marker), Lane 1 (Swat), Lane 2 (Peshwar), Lane 3 (Kohat), lane 4 (Rawalpindi), Lane 5 (Lahore) and Lane 6 (Dera Ismail Khan).

Table 3. Pigmental analysis of different costar oil accessions collected from different climatic zones.

S. No.	Accessions	Climatic Zones	Chl <i>a</i> (mg/g fw)	Chl <i>b</i> (mg/g fw)	Total Carotenoids (mg/g fw)
01	L1	Swat	2.39	9.39	1721.90
02	L2	Peshawar	4.27	16.67	1489.01
03	L3	Kohat	9.35	56.40	2097.36
04	L4	Rawalpindi	5.69	14.45	1396.12
05	L5	Lahore	8.39	3.65	1248.24
06	L6	Dera Ismail Khan	3.17	46.64	1006.57

Discussion

Climatic circumstances have a momentous effect on the biological and molecular aspects of medicinal flora, as worldwide climatic variations due to various man made activities distresses diverse plant species increasing plant transience and extension (IPCC, 2013). The present study was conducted to observed biochemical and molecular characterization of costar oil plant collected from different climatic zones of Pakistan on the basis of their seed storage proteins and cell membrane embedded proteins. According to our results all the accessions from different zones showed no such variations in there protein banding pattern, and very light variation was noted in the leaf extracted proteins in terms of the intensity of the polypeptides collected from different climatic zones of Pakistan. Low variations in both polypeptide intensity and presence of polypeptides was in closed agreement with the findings of Kourand Singh (2004) and Cheema et al. (2010) , they also observed three zones of banding profile. The current analysis publicized very inadequate discrepancy in castor bean collected from different climatic zones of Pakistan. Likewise, genotypes from diverse ecological niches did not fluctuate in protein types. As genetic multiplicity for a plant species is vital for its perfection and its genetic variations. Different tools are used

to find out these disparities among which RAPD has a reliable technique to find genetic variations (Kapeteyn and Simon 2002). We observed no such polymorphism with four primers used, between different accessions of different climatic zones, so this is clear that RAPD is not suitable for finding genetic variations caused by environment. It is due to the fact that unlike or biochemical parameters DNA polymorphisms are not affected when exposed to diverse environmental conditions.

Photosynthesis is a sensitive process and can affect plants that have been exposed to different environmental stresses like high temperature etc. (Camejo et al., 2005). Process of Photosynthesis is affected by various environmental factors like temperature, light interval, presence of humidity and CO₂ concentration, which directly or indirectly affects plant physiochemical and molecular processes. As photosynthesis is often inhibited at both low and high temperatures (Berry and Bjorkman 1980). The lower level of photosynthetic pigments in plants collected from colder region i.e. Swat where the lower temperature render the photosynthesis and pigment formation. Similarly, the results also showed that plants collected from Dera Ismail Khan possesses low level of photosynthetic

pigments which can be attributes as at higher temperature. According to Xu et al. (1995) and Dekov et al. (2000), plants exposed to high temperature stress, a disruption in the form, structure and function related to chloroplasts and reduction in the accumulation of chlorophyll can be occurred.

Conclusion

It is concluded that, differentiation of the closely related accessions/cultivars of castor bean on the basis of seed storage proteins under diverse environments is difficult. The present investigation revealed no variation with reference to their total seed protein profiling in different accessions grown under different environmental conditions. RAPD analysis indicated same banding pattern which showed that among the all the accessions no variation was found in their genomic DNA. Present study also suggested that the level of photosynthetic pigments in different plants collected from various climatic zones were also different.

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