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Variation in Biochemical Properties in Seeds of *Jatropha curcas* Linn.: a Study of Hilly Regions of Uttarakhand

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ABSTRACT
Jatropha curcas has recently come into prominence as source of bio-diesel. This species has naturalized stands spread all over the country and due to varied climatic conditions is likely to have a high degree of variability. In view of this, biochemical and electrophoretic analysis in J. curcas was studied in seven seed sources collected from Central Himalayan zone. In general all seed sources showed variation in soluble protein, carbohydrate and total amino acids content. Considerable differences in the quantity of individual polypeptides as displayed by the density of bands, was found among the seed sources. Further, to determine the similarity in protein profile between different seed sources coefficient of similarity was worked out. Among all the seed source Kalachauna and Saknidhar had highest similarity of 100% whereas Gandhari had the lowest ranging from 42 to 57%. The UPGMA identified Saknidhar, Kalachauna, Naithana and Basnal in one group, Basnal and Dhruvpur in another group and Gandhari as a distinct seed source. Five esterase isoenzymes and 3 each of acid phosphatase and peroxidase were recorded in the seed sources of which more variation between seed sources were in esterase than in rest two enzymes. Kalachauna and Dhruvpur had 100% similarity sharing all the isoenzymes present whereas Sakanidhar and Naithana also had 100% similarity but had 1 isoenzymes of esterase lacking. As compared to esterase and peroxidase the isoenzyme pattern of acid phosphatase was quite similar in all the seed sources of J. curcas studied here.
Key words: *Electrophoresis, genetic diversity, seed source and isoenzymes.*

INTRODUCTION

Jatropha curcas L., a species of family Euphorbiaceae, has recently come into prominence as a potential source of bio-diesel not only because of the promising oil characteristics favouring its use as liquid fuel but also because of the unique characteristics of the plant habit and habitat preferences. In fact, depleting fossil fuel reserves and the environmental implication of usage of conventional energy forms has evoked worldwide interest in exploring new, renewable and environment friendly sources of energy. The oil seed crops are one such resource, of which the non-edible oils hold more promise being non-competitive to the food crops. The oil obtained from the seeds of *J. curcas* has been reported to be an efficient substitute fuel for diesel engines and therefore the focus on this species. Variability studies are the prerequisite and of paramount importance for developing tree improvement strategy. Genetic diversity among the seed source can also be assessed by the using polypeptides and isoenzyme variability. In many cases the correct identification of the plant species may difficult, so for over come this problem electrophoretic techniques may help in identification of the cultivars properly. Electrophoretic analysis of proteins and isoenzyme offers an efficient and cost effective method towards evaluation of geographical and taxonomic distribution of genetic variation for sampling strategies in germplasm conservation [7]. Isoenzymes are valuable markers for cultivar identification and varietal purity tests in seeds lots [8, 21, 2-20]. Despite having such importance, studies on the variability at of these species at seed source level are scanty. Therefore, in this paper, an initial assessment of population variability, in terms of genetic (SDS-PAGE and isoenzymatic) and quantitative (Soluble sugar, starch content, soluble proteins and free amino acids) variation is presented.

MATERIAL AND METHOD

Collection of seed samples: Seeds of *J. curcas* were collected from 7 geographical locations of Central Himalaya varying in altitude from 550 to 1050 m asl (Table 1)

Biochemical analysis: The method described by Bradford [6] was used for quantitative estimation of protein. Soluble sugars and starch were estimated following the method described by [17]. Total free amino acids were estimated according to method of [19].

SDS-PAGE analysis: Two gram seeds of *J. curcas* were homogenized in 10 mL of defatting solution containing (2:1) chloroform and methanol. To estimate the protein seed homogenates were centrifuged at 10,000×g for 20 min. Supernatant was discarded and pellet was kept in fridge overnight. The pellet was suspended again in defatting solution, homogenized, centrifuged and supernatant discarded. This process continued until the fat was completely removed from the sample. Total soluble proteins were extracted by homogenizing the 100 mg defatted tissue with 0.1 M tris–HCl [{2–Amino–2–(hydroxymethyl) propene–1, 3–diol}–Hydrochloric acid] buffer pH 7.5 with 0.5% β–merccaptoethanol and 1% PVP (Polyvinylpyrollidone). A pinch of protease inhibitor PMSF (Phenylmethylsulphonyle fluoride) was added at the time of grinding to minimize the proteolytic activity. Bradford method was used for quantitative estimation of proteins for loading equal amount of protein on the gel. Polypeptides were separated by SDS–electrophoresis using the method of [16]. Standard protein (GENEI) of molecular weight ranging from 6.5 kDa to 97 kDa was simultaneously run along with sample. The gel was run at 20 mA in electrophoresis apparatus mini model protein (BIORAD). The gel was stained with coomassie brilliant blue and destained with destaining solution (10 mL of acetic acid and 40 ml of methanol and final volume made to 100 mL with distilled water) and preserved in 7% acetic acid.

Isoenzymes analysis: For isoenzymes study germinated seeds (at the stage of radicle emergence) were used. One gram germinated seeds tissue was crushed in liquid nitrogen and kept at -20°C. This tissue was homogenized in 0.1 M tris–HCl [{2–Amino–2–(hydroxymethyl) propene–1, 3–diol}–Hydrochloric acid] buffer pH 7.5 with 0.5% β–merccaptoethanol and 1% PVP (Polyvinylpyrollidone). Isoenzymes of Esterase, Peroxidase and Acid Phosphatase were analyzed (on 10% polyacrylamide slab gels) according to the methods described previously [5, 10] and were separated and detected by method of [24].

Statistical analysis: For quantitative observation appropriate replications were made and mean with standard deviation was used. The data were statistically analyzed using Analysis of Variance (ANOVA) with least significant difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

Biochemical variability: Comparative biochemical analysis of seeds from different seed sources of *J. curcas* showed distinct variation in terms of total carbohydrates, starch, soluble protein and total free amino acids (Figure 1a-d). The differences among the seed sources were statistically significant. Total soluble sugars content was recorded maximum (61.68 mg g^{-1}) in Kalachauna seed source and minimum (23.65 mg g^{-1}) in Naithana seed source showing a high degree of variation as the differences between these sources was of nearly 65%. Seed source variation of different magnitude in carbohydrate has been found in other species at varietal or genotypes level. A study done by [22] in desi chickpea (*Cicer arietinum* L.) varieties cultivated in arid zone of Pakistan indicates that the total carbohydrates in these varieties varied from 64.90±0.16g/100g to 66.51±0.11g/100g showing a narrow range of variation even at varietal level. Contrary to this, biochemical constituents of some promising sesame (*Sesamum indicum* L.) genotypes were studied by [1] who reported that variations in soluble sugars ranged from 5.81% to 12.19% among genotypes indicating a high level of variation at the level of genotypes.

The variation in soluble protein content between the maximum at Gandhari (170.60 mg g^{-1}) seed source and minimum at Naithana (112.00 mg g^{-1}) seed source was about 35% which is highly significant variation. In fact variation of this magnitude has been reported earlier from wider range of distribution of this species. A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *J. curcas* germplasm from different countries has been done by [3] who observed variation in crude protein among all the accessions ranging from 18.8 to 34.5%. Recently [12] studied agroclimatic conditions, chemical and nutritional characterization of different provenances of *J. curcas* from Mexico and reported that variation in the contents of crude protein in kernels among different provenances was in the range of 19 to 33%. These findings are in agreement with the results obtained in present study.

Table 1: Different geographical locations of *J. curcas* taken for study

Seed source	District	Elevation (m)	Latitude (°N)	Longitude (°E)
Naithana (NTH)	Tehri	550	30°13'633"	78°46'333"
Dhruvpur (DPH)	Pauri	650	29°25'103"	78°35'122"
Bamoth (BT)	Chamoli	787	30°16'267"	79°10'250"
Saknidhar (SDH)	Tehri	828	30°05'420"	78°32'240"
Kalachauna (KCH)	Almora	940	29°48'443"	79°16'848"
Gandhari (GDH)	Rudraprayag	970	30°18' 902"	79°02'422"
Basnal (BS)	Almora	1024	29°52'470"	79°18'608"

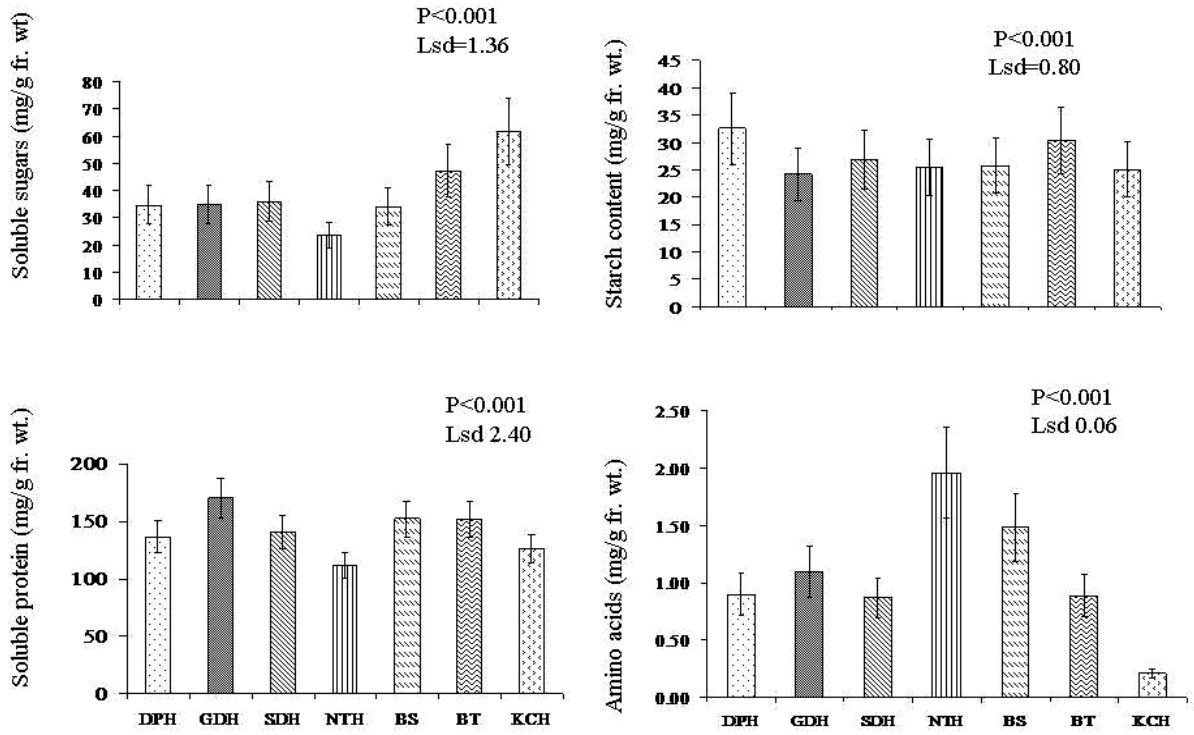

Figure 1. Biochemical variation in different seed sources of *J. curcas*.

SDS PAGE profile: The variation in polypeptides pattern of different seed sources of *J. curcas* has been shown in Figure 2. However, all bands resolved on the gel are not prominently seen in the photographs. Two seed sources, Kalachauna and Saknidhar had the highest number of polypeptides that were common in both the sources. Three other seed sources Dhruvpur, Naithana and Bamoth had 13 bands each followed by BS with 10 bands and only 4 bands were recorded in GDH seed source that were common with all other seed sources. All 13 bands in Dhruvpur, Naithana and Bamoth were not common to each other. Only 11 bands were common in Bamoth and Naithana as well as Bamoth and Dhruvpur whereas Dhruvpur and Naithana shared 12 bands. As evident in the photographs also considerable differences in the quantity of individual polypeptides, as displayed by the density of bands, was found among the seed sources. However, band number 1 of 97 kDa that was common in all seed sources, also had same intensity. Contrary to this, although the polypeptide bands marked 4, 5 and 9 in the photographs were common in all the seed sources, intensity of the bands varied indicating the quantitative differences between the sources. To further determine the similarity in protein profile between different seed sources coefficient of similarity was worked out. Among all the seed sources Kalachauna and Saknidhar had highest similarity with other sources whereas Gandhari had the lowest.

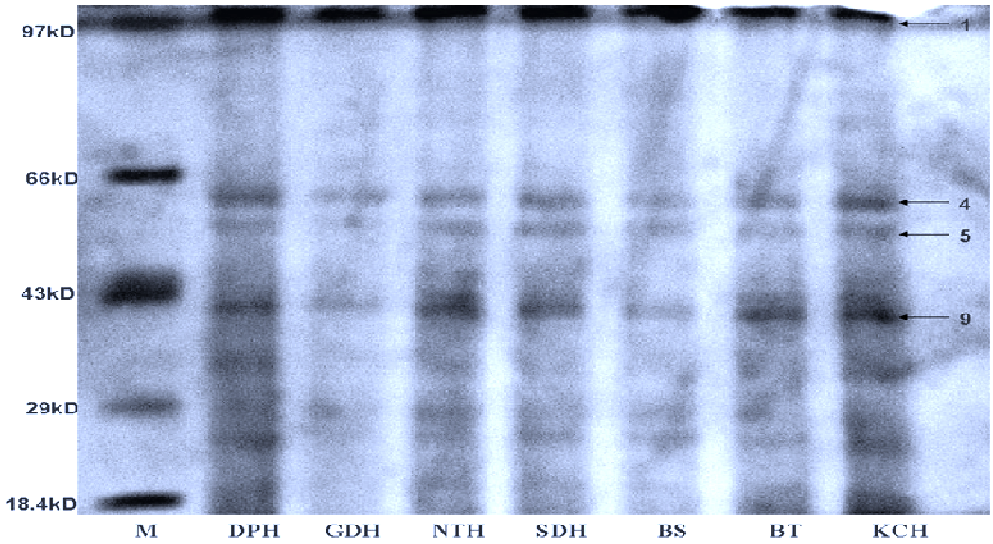

Figure 2 Polypeptide band pattern in different seed sources of *J. curcas.*

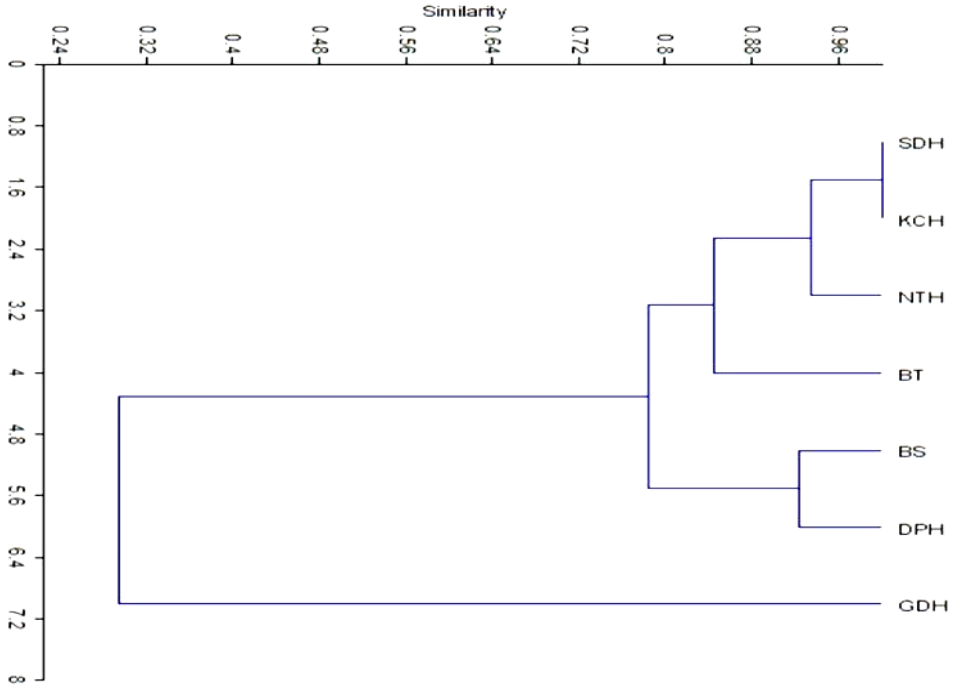

Figure 3: Polypeptide-based unweighted pair group method (UPGMA) dendrogram for 7 seed sources of *J. curcas*.

In order to determine the genetic similarity among the seed sources, cluster analysis was also performed based on the analysis of polypeptides banding pattern of different protein markers the seed sources. The similarity matrices after Jaccard, Sokal and Michener [13, 23] produced dendrograms with the identical pattern of different seed sources. The dendrogram analysis of different seed sources distinguishes two sub clusters and one line (Figure 3). One sub cluster consists of seed sources Saknidhar, Kalachauna, Naithana and Bamoth and other sub cluster is formed of seed sources Basnal and Dhruvpur. It should be noted that the grouping within sub clusters occurs at small genetic distances. The line which consists of seed source Gandhari is loosely linked to the above clusters.

Significant implications of SDS–PAGE techniques in elucidating species diversity, their characterization and identification have also been demonstrated in many other species [18, 9, 4, 24-11].

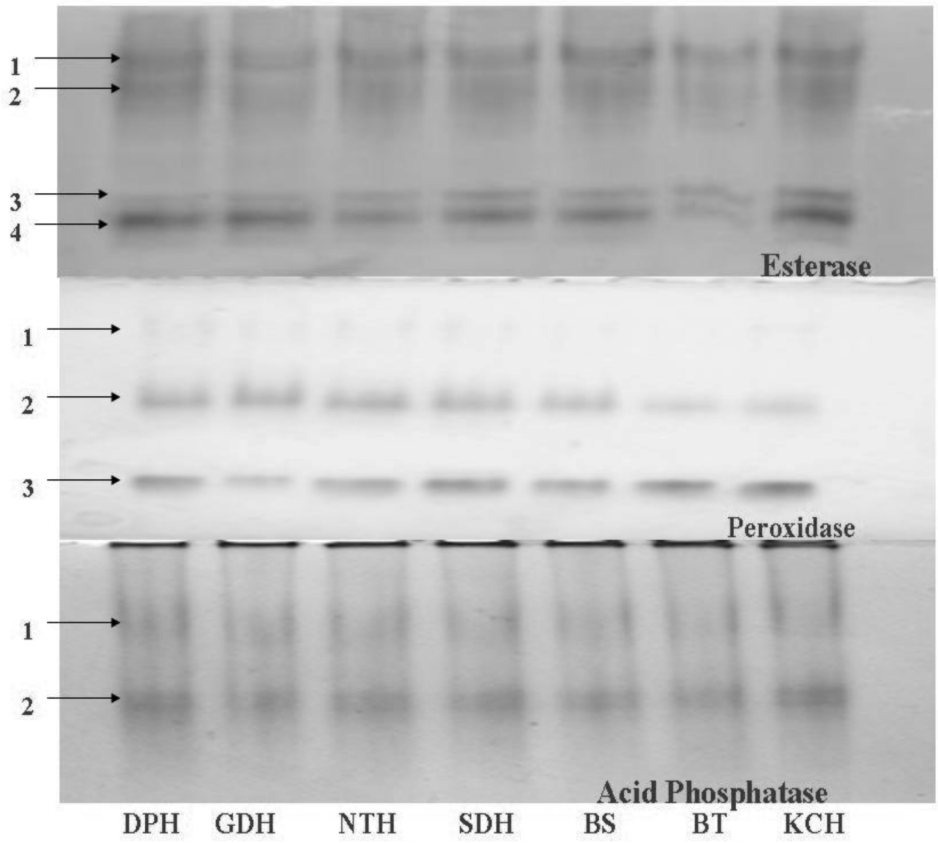

Figure 4: Isoenzymes-based unweighted pair group method (UPGMA) dendrogram for 7 seed sources of *J. curcas*.

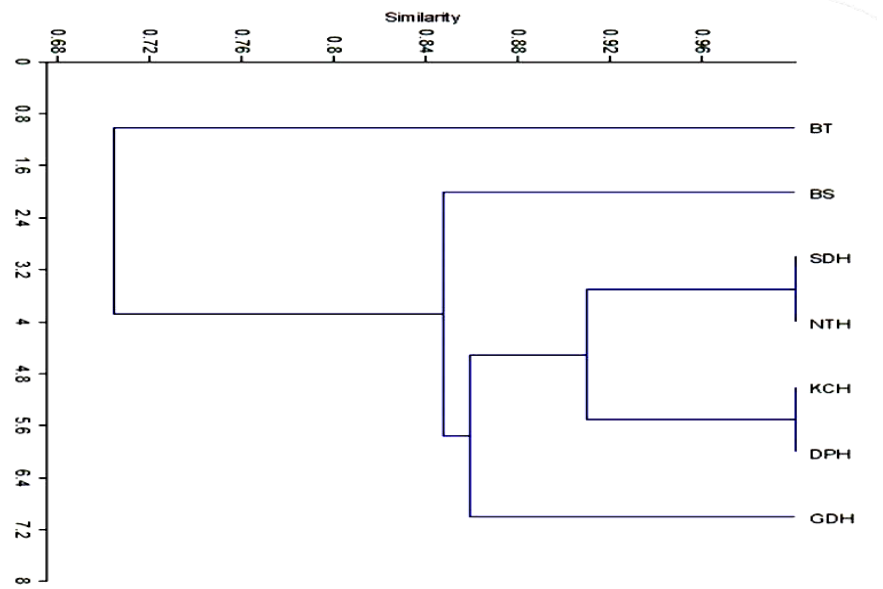

Figure 5 Isoenzymes-based unweighted pair group method (UPGMA) dendrogram for 7 seed sources of *J. curcas*.

Isoenzymes: Similar to polypeptides all the bands of esterase isoenzymes are not prominently seen in the photograph. A total of 5 bands of esterase isoenzymes were observed in the seven seed sources of *J. curcas* of which three were common to all but band number 2 was absent in Gandhari and Bamoth seed source whereas band number 5 was present only in Dhruvpur and Kalachauna seed sources. The intensity of bands also differed considerably among the seed sources.

Pattern of peroxidase isoenzymes has also been shown in plate 4. Total three bands appeared on the gel among all the seed sources. Band number 1 appeared only in Dhruvpur, Sakanidhar, Naithana and Kalachauna with low intensity and was absent in others. Band number 2 appeared in all the seed sources but intensity was different i.e. Dhruvpur, Gandhari, Naithana and Saknidhar seed sources showed high intensity and Bamoth and Kalachauna seed source showed very low intensity. Band number 3 was also present in all seed sources but high intensity of the band was recorded in Kalachauna, Bamoth, Saknidhar, Dhruvpur and Naithana seed sources and low intensity was recorded in Gandhari seed source.

Pattern of acid phosphatase isoenzymes has also been shown in figure 4 wherein a total of 3 bands were noticed of which two bands were common in all the seed sources. Band number 1 appeared with high intensity in Dhruvpur seed source and low intensity in Gandhari seed source. Band number 2 was absent only in Bamoth and present in rest all other seed sources. Thus as compared to esterase and peroxidase, the isoenzyme pattern of acid phosphatase was quite similar in all the seed sources of *J. curcas* studied here.

In isoenzymes banding pattern (*viz*. esterase, peroxidase and acid phosphatase) only 5 bands in case of esterase and 3 bands each in case of peroxidase and acid phosphatase were present among all the seed sources and also the banding pattern had lower dissimilarities therefore the dendrogram analysis were recorded on the basis of pooled data. Isoenzymes-based unweighted pair group method (UPGMA) dendrogram for 7 seed sources of *J. curcas* showed one sub cluster and two loosely linked lines, which consist of Basnal and Bamoth. Sub cluster consists of seed sources Saknidhar, Naithana, Kalachauna, Dhruvpur and Gandhari (Fig. 4).

Among three isoenzymes total 5 bands were recorded of which 3 were common in all the seed sources but band number 2 was absent in Gandhari and Bamoth seed sources whereas band number 5 was present only in Dhruvpur and Kalachauna seed sources and none else. Out of 3 isoforms of peroxidase recorded band number 1 was absent in Gandhari, Basnal and Bamoth and rest four seed sources shared all the isoforms. Further narrower differences were recorded in case of acid phosphatase in which only Bamoth seed source had band number 2 missing and rest all shared all the isoforms. However, similar to protein the intensity of bands varied considerably among the seed sources indicating more quantitative than qualitative variation. Provenance variation in the isoenzymes has been reported earlier by several workers wherein the magnitude as well as pattern of variation differed with species. In some cases attempt was made to establish relationship seed source variation with the geographical distribution of the seed sources. For example, a study on the isozymes of peroxidase and esterase in the seeds of eleven provenances of Chinese fir (*Incunninghamia lanceolata)* with distinct latitudinal differences was undertaken by [26] who observed considerable variations among the geographical provenances but the dissimilarities had no relation with latitude. Suitability of seed esterases for establishing distinctness, uniformity and stability of pearl millet genotypes was studied by [15] who reported that a significant difference in esterases in pearl millet seeds existed that led to differentiation of genotypes in distinct groups. Distinct isozyme pattern of peroxidase and acid phosphates was found in teak provenances. Kertadikara, and Prat [14] studied genetic structure in Teak (*Tectona grandis* L. f.) provenances and observed the variation in the activity of acid phosphatase and peroxidase enzyme among provenances. They found two regions of teak were highly polymorphic in acidic phosphatase and one region appeared for peroxidase enzyme patterns. Welter [25] also studied electrophoretic variation in esterase, peroxidase and acid phosphatase in some native Greek taxa and based on the similarities suggested phytogenitic relationship. Isoenzymes-based unweighted pair group method (UPGMA) dendrogram for 7 seed sources of *J. curcas* showed one sub cluster and two loosely linked line, which consist of Basnal and Bamoth. Sub cluster consists of seed sources Saknidhar, Naithana, Kalachauna, Dhruvpur and Gandhari (Figure 4). Dissimilarities

of different magnitude among the seed sources possibly indicate their genetic diversity which could have originated due to their discrete location prohibiting gene flow between the seed sources.

CONCLUSION

The present study revealed that among all the seed sources of *Jatropha curcas* collected from various altitudes, Kalachauna and Saknidhar had highest similarity whereas Gandhari had the lowest. The important point that emerged from the present study is that magnitude of variation among the seed sources was at par with those reported from a wider geographical range from other parts of the country. This could possibly be due to wide range of habitat diversity at shorter distances in the study area, a characteristic of the mountain region, and/or also due to genetic diversity in the seed sources owing to their discrete location prohibiting gene flow between the seed sources.

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