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Research Article

# Studies on genetic variability of ecotypes and Biochemical analysis of wild edible medicinal tree Lasuda (*Cordia myxa* L.) in Himalayan Shivaliks

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**Abstract:** *Cordia myxa* L. is traditional medicinal tree with multiple uses such as vegetable, fodder and soil binder tree and used as delicacy in rural cuisine in summer and rainy season. The present study was an attempt about genetic variability, heritability and genetic advance among the important traits of lasuda crop and use this by modern techniques to develop highly nutritive strains. The overall values of PCV were higher than values for all the characters in among all the landraces under study. PCV was highest for fruit diameter (32.95) followed by seed diameter (30.92), pulp weight (22.75) and fruit weight (17.01), while, lowest level of PCV was observed for fruit length (8.62). Almost similar variations were noted in GCV, highest GCV was found for fruit diameter (32.47) followed by seed diameter (28.54), pulp weight (21.02) and fruit weight (15.57), while, the lowest level of GCV was observed for fruit length, which was found to be 7.77). The estimate of genetic advance was highest for fruit weight (531.11g) followed by fruit diameter (18.85). Whereas, lowest genetic advance was observed for fruit length (4.49) and pulp weight (5.62).highest genetic gain was recorded in the fruit diameter(65.91 followed by seed diameter(54.27), pulp weight (40.01) and fruit weight(29.34) and lowest genetic gain was recorded in fruit length (14.45). The HCA analysis based on the ward's method distance computed clustered the strains into five homogenous groups. The results further revealed that the TSS content varied from 3.19-5.167. The pH varied from 6.33–6.73 among different ecotypes. The titratable acidity varied from 0.793 – 1.690 per cent among all the ecotypes, Total protein ranged from 6.56 to 11.41 per cent. The moisture content ranged from 77.33 to 91.00 per cent. The ascorbic acid contents 25.77-36.72 and the total sugar contents varied from1.270-1.993 percent ranged. The estimates of PCV and GCV were high which indicate that selection can be effective for the improvement of these traits as the variability between genotypes for these traits was highly heritable.

**Keywords:** *Cordia myxa* L; genetic variability; biochemical; Shivaliks; Himalayas.

## Introduction

*Cordia myxa* L. (Boraginaceae)is traditional underutilized fruit tree with multiple uses such as vegetable, fodder and soil binder tree of lower hill regions of Himachal Pradesh. It is a delicacy on rural "Pahadi" cuisine in summer and rainy season. Mostly used for pickle making and dried for its use in local "Badian" an off-season vegetable of pulse floor and *Cordia myxa* fruit mix. It extends from Jammu, Himachal Pradesh to Uttarakhand and in dry part of Rajasthan of Indian Subcontinent. (Meghwal, 2007).

*Cordia myxa* contains Stigma-sterol up to 5.86 percent that prevents certain types of cancers including ovarian, prostate, breast, and colon cancers, Possess certain antioxidant, hypoglycemic, hyperlipedimic and thyroid inhibiting properties (Shwaish and Al-Imarah, 2017; Rahman *et al.*, 2016). *Cordia myxa* has certain analgesic, anti-inflammatory, immune

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modulatory, antimicrobial, anti-parasitic, insecticidal, cardiovascular, respiratory, gastrointestinal and protective effects (Al-Snafi, 2017; Anjaria *et al.,* 1997). The importance of this fruit is well recognized both in rural as the tender mature fruits are mostly pickled and also used for vegetable purpose. The fruits of *C. myxa L.* (Lasuda fruit) have medicinal features and are considered as anthelmintic, diuretic, demulcent and expectorant (Al-Awadi *et al.,* 2001; Alami and Macksad, 1974; Patterson and Williams, 1976). They are rich source of carbohydrates, phosphorous, Sugar and calcium (Ozha and Kulkarni, 2017). The fruits of *C. myxa* can be easily dried after blanching for use during off season which can give good returns even under rainfed marginal and less productive areas of hills. This species plays an important role in the rural economy of arid regions (Peter, 2007).

Study and evaluation of the variation is the first step for any breeding program and tree breeding depends on the existing variability in the nature under different edaphic-climatic conditions. The Himalayan region is rich in its biodiversity under natural habitats where seedling trees come up in plethora naturally. Very scanty work on morphological variation in fruit quality and conservation has been reported on (*C. myxa*) Lasuda genotypes in this region. The evaluation of existing germplasm in nature followed by selection and preservation of germplasm in field gene banks will be helpful in screening the potential of *Cordia myxa* which is in the way of utilization of the untapped potential of the wild germplasm for mankind. A good knowledge of genetic wealth might also help in identifying desirable cultivars for commercial cultivation (Korir *et al.,* 2012).

The present study was an attempt to describe the variability in selected Lasuda genotypes and to identify the most useful variables for discrimination among genotypes, and detect relationships among genotypes, gather information about genetic variability, heritability and genetic advance among the important traits of Lasuda (*Cordia myxa*) crop.

## Materials and Methods

A survey to explore the population of *Cordia myxa* L. (Lasuda) in lower Shivalik ranges of Himachal Pradesh were undertaken during two consecutive years (2018-2020) to screen out the ecotypes with higher productivity from wild (Table-A). The sampling procedure included the delineation of the agricultural farms and forest area in the entire Himalayan Shivalik range depending upon the landraces variability with a wide variation in the morphological characters.

The survey was conducted at twenty sites in the different regions along altitudinal and longitudinal range and out of those seven ecotypes were considered for further study due to its superiority in visual phenotypic characters for their fruits and productivity (Table-A). Naturally growing trees of *Cordia myxa* from each was selected out of the over 15-20 trees on the basis of regular fruit production and observed phenotypic diversity. Selections of genotypes were made according to relevant morphological traits of the tree. The selected trees were marked and fruit sampling was done from each site for measuring observations on various physico-chemical traits.

Fruit weight was recorded using an electronic balance with instrument sensitivity of 1 mg. Fruits/bunch was calculated by counting the total number of fruits in each bunch, in the marked shoot. Hundred fruits from each of the identified trees were collected from all the four orientations of the tree, i.e. 25 from north, 25 from south, 25 from east and 25 from west. Weight of 25 fruits was divided by total number of fruits to obtain the average fruit weight. Pulp

weight of 25 selected fruits per tree was estimated by deducting the stone weight from the total fruit weight. Thereafter pulp: stone ratio was obtained by dividing the pulp weight by stone weight and average was calculated. Total soluble solids content and acidity was determined as described by AOAC (1995). Genotypic and phenotypic coefficient of variation was calculated according to Singh and Chaudhary (1985). Genetic advance (GA) and genetic advance percent (GA%) were estimated as proposed by Johnson, *et al*. (1955) and Allard (1999).

Heritability estimate in the broad-sense ($h^2$) on mean basis for each location and combined locations was used as suggested by Eckebil *et al*., (1999).

$$\text{Heritability } (h^2) = \frac{\sigma^2 g \times 100}{\sigma^2 p}$$

Where $\sigma^2 g$ is: genotypic variance of the population & $\sigma^2 p$ is phenotypic variance of the population

Genotypic coefficient of variation (GCV) was calculated using the following formula as suggested by the Burton (1952).

$$\text{GCV} \quad \frac{\sqrt{Vg}}{\bar{X}} \text{X100}$$

Where, Vg = Genotypic variance & $\bar{X}$ = Germplasm mean

Phenotypic coefficient of variation (PCV) was calculated using the following formula as suggested by Burton (1952).

$$\text{PCV} \frac{\sqrt{Vp}}{\bar{X}} \text{X 100}$$

Where, $Vp$= phenotypic variance& $\bar{X}$ = Germplasm mean.

The data in the two seasons were subjected to statistically analyzed according to the technique of analysis of variance (ANOVA) for the alpha lattice design developed by Patterson and Williams (1976) The data obtained were subject to analysis of variance using randomized block. The 7 sites were considered as treatment and the individual selected tree at each site was taken as the replication. The coefficient of variation for fruit quality was calculated.

The samples were subjected for biochemical analysis as recommended by AOAC (1990) for parameters *viz*., pH, TSS, Ascorbic acid, Total Acidity, Moisture content, nitrogen and protein.

The pH of different fruit waste samples was measured using digital pH meter (analog model).

Total soluble solids of fruit waste samples were determined using digital Refractometer.

The titrable acidity was determined by as per the procedure followed by Srivastava and Kumar (Srivastava and Kumar, 1993).

Crude protein was calculated by multiplying with a conversion factor of 6.25 (AOAC, 1984).

**Table A**: Description of Seed Sources of *Cordia myxa* under study

| Place | Tree Height(m) | Tree Diameter(cm) | Latitude | Longitude | Altitude(m) |
|---|---|---|---|---|---|
| Dehra | 11.5 | 32.75 | 31.88 N | 76.21 E | 607 |
| Gumer | 12.3 | 25.89 | 31.87 N | 76.32 E | 560 |
| Lappiana | 11.5 | 36 | 32.17 N | 76.08E | 579 |
| Raja Talab | 13.4 | 37.9 | 32.19N | 75.90E | 493 |
| Gangath | 15.6 | 41 | 32.23E | 75.82E | 434 |
| Shahpur | 18.4 | 36.9 | 32.22N | 76.18E | 602 |
| Thana | 12.8 | 32.34 | 32.20N | 75.70E | 501 |

## Results and Discussion

The study of quantitative and qualitative traits confirmed that all ecotypes are highly adapted to the environmental conditions and results of this study indicated high morphological diversity among *Cordia myxa* ecotypes. The results exhibited in Table-1 revealed variation in fruit weight (1725.45 g to 2092 g/100 fruits) was found in Shahpur to Suliali Ecotype. The pulp weight ranged from (10.22 g-18.767 g per fruit) in Raja Talab to Suliali ecotype. Seed diameter varied from (8.297-17.017 mm) in Suliali to Raja talab ecotypes. Fruit diameter varied from 21.33-36.52 g in Gumer ecotypes to Suliali ecotypes and fruit length ranged from (23.90 mm-34.63 mm) in Gumer ecotype to Suliali Ecotype. If we study the overall physical parameters of the fruits, we can conclude that Suliali, Lappiana and Dehra ecotypes were superior in the quantitative characters than other ecotypes. The findings were in accordance with findings of Bhatnagar *et al.,* (2015) but the values were much higher than the studies conducted by then. This may be due to the reason that the Himalayan climate have its influence on the species growing in it for long.

The wide spectrum of variability for all characters provides greater opportunity for the isolation of best genotypes to be fitted in breeding program. The analysis of variance for all the quantitative fruit characters revealed that treatments differences were highly significant indicating the presence of inherent genetic differences in our experimental material (Table-2).

*Coefficient of variation*: The variability parameters like phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) indicated the presence of considerable variations for all the characters under study among all the ecotypes. The overall values of PCV were higher than GCV values for all the characters in among all the landraces under study (Table 2). PCV was highest for fruit diameter (32.95) followed by seed diameter (30.92), pulp weight (22.75) and fruit weight (17.01), while, lowest level of PCV was observed for fruit length (8.62). Almost similar variations were noted in GCV, highest GCV was found for fruit diameter (32.47) followed by seed diameter (28.54), pulp weight (21.02) and fruit weight (15.57), while, the lowest level of GCV was observed for fruit length, which was found to be 7.77. Bhatnagar *et al.,* (2015) have noted similar kind of variations in *Cordia myxa* in the Jhalabar district of Rajasthan but with lower magnitude. He further coined that high heritability effects suggest dominant epistasis and high inheritance of fruit characters from one generation to another generation.

The difference between the values of PCV and GCV of characters indicated impact of environmental factors on the expression of these traits but the lower difference in values between of PCV and GCV, indicated that environment do affect the fruit characters but not very high. So, we can use these characters for improvement of the crop productivity by breeding under different climates. The Knowledge of nature and magnitude of genetic variability present in the population is of immense value for planning efficient breeding program to improve the yield potential of genotypes. The extent of variability as measured by PCV and GCV provides information regarding the relative amount of variation in different characters (Kumar *et al.,* 2015).

*Heritability*: The estimate of broad sense heritability is the proportion of total genetic variance involving both additive and non-additive types to total phenotypic variance. The estimates of broad sense heritability (h2) were high almost all the characters under study it ranged between the highest value of heritability was found in the fruit diameter (97.11%)

followed by pulp, Seed diameter (85.22), 100 fruit weight (83.71%) and lowest in fruit length(81.41). Most of the traits included in the present investigation were having very high broad sense heritability across among all the landraces. This indicated that all characters are less influenced by the environmental conditions and selection for such characters on the basis of phenotype will be effective. Similar findings of heritability for grain yield and other characters have also been reported by Shanthi *et al.,* (2011), Badawy (2012) and Kumar *et al.,* (2015) in agricultural crops.

The estimate of genetic advance found to be high for fruit weight (531.11) followed by fruit diameter (18.85). Whereas, lowest genetic advance was observed for fruit length (4.49) and pulp weight (5.62). Highest genetic gain was recorded in the fruit diameter (65.91) followed by seed diameter (54.27), pulp weight (40.01) and 100 fruit weight (29.34) and lowest genetic gain was recorded in fruit length (14.45) (Table2). The obtained results are in accordance to similar findings with some deviations of Badawy (2012) and Bekele and Rao (2014) in agricultural crops.

Analysis of data in Table.3 depicts results of fruit biochemical contents of all ecotypes were significantly variable. The results revealed that the TSS (Total Suspended Solids) content was observed maximum in the Thana collection to the tune of 5.167 and lowest TSS was found in Raja talab (3.19). This could be due to the presence of higher pulp in the Thana ecotypes than other ecotypes.

The pH varied from 6.33–6.73 among different ecotypes showing all the ecotypes fruit has acidic to neutral pH. The titratable acidity varied from 0.793–1.690 per cent among all the ecotypes. However, more acidity was in the Shahpur collection than all other types.

The proximate composition of different ecotypes revealed that the total protein ranged from 6.56 to 11.41 per cent. The moisture content ranged from 77.33 to 91%.

This shows that all the fruits have good moisture contents which make them excellent for vegetable and for other pickle making. The ascorbic acid contents25.77-36.72 and the total sugar contents ranged from 1.270-1.993%. Shwaish and Al-Imarah (2017) reported the similar biologically-active ingredients in *Cordia myxa* and found between 3-8%, except the fat-soluble form of ascorbic acid which accounted for around 10% of the identified portion. *Cordia myxa* fruit is regarded as a good source for fibers, proteins and carbohydrates, and some studies suggest promoting the fruit as a carbohydrate and protein supplement for cereal-based diets in poor rural communities (Al-Snafi, 2017).

A hierarchical clustering (HCA) was performed on the basis of evaluated quantitative traits *viz.,* fruit seed and plant characters. In this analysis, 7 ecotypes were put under test to establish the relationship among them. The HCA analysis based on the ward's method distance computed clustered the strains into five homogenous groups (Figure 1). The cluster 1 was composed of Lappiana, Gangath and Shahhpur ecotypes. The second cluster consisted of Dehra. The third cluster consisted of Suliali and the fourth one was Gumer Ecotype and fifth cluster was of Raja Talab ecotype. The analysis further suggests that Lappiana, Gangath and Shahpur ecotypes had similarities whereas Dehra, Suliali (Thana), Gumer and Raja Talab ecotypes were associated at higher level of clustering. Fruit weight and seed diameter and seed weight were negatively correlated (Table-4) whereas fruit diameter and pulp weight were significantly and positively correlated with the fruit weight of the different ecotypes.

**Table 1**. Analysis of variance for important morphological characters of *Cordia myxa* ecotypes.

| Treatment | 100 fruit weight(g) | Pulp weight (g)/ per fruit | Seed Diameter (mm) | Fruit Diameter (mm) | Fruit length (mm) |
|---|---|---|---|---|---|
| Dehra | 2,089.33 | 16.15 | 10.697 | 33.733 | 33.9 |
| Lappiana collection | 1,794.10 | 13.587 | 11.707 | 33.667 | 29.567 |
| Suliali collection | 2,092.67 | 18.767 | 8.297 | 36.52 | 34.63 |
| Gangath collection | 1,856.00 | 13.633 | 9.64 | 28.43 | 28.867 |
| Shahpur collection | 1,725.45 | 11.95 | 14.45 | 28.597 | 29.76 |
| Raja talab | 1,804.67 | 10.22 | 17.017 | 21.69 | 29.70 |
| Gumer | 1880.36 | 10.5 | 9.17 | 21.33 | 23.90 |
| MEAN | 1,808.94 | 13.54 | 11.71 | 29.14 | 30.05 |
| C.D. | 229.093 | 2.253 | 2.657 | 2.952 | 2.127 |
| SE(m) | 71.77 | 0.706 | 0.832 | 0.925 | 0.666 |
| SE(d) | 101.506 | 0.998 | 1.177 | 1.308 | 0.942 |
| C.V. | 6.867 | 8.7 | 11.887 | 5.6 | 3.72 |

**Table 2**: Heritability, genetic advance, genotypic and phenotypic coefficient of variation for important characters of *Cordia myxa*

| Genetic parameters | 100 fruit weight (g) | Pulp weight (g)/per fruit | Seed Diameter (mm) | Fruit Diameter (mm) | Fruit length (mm) |
|---|---|---|---|---|---|
| ENV Variance | 15455.27 | 1.495 | 2.078 | 2.566 | 1.332 |
| Genotypic variance | 79409.33 | 8.72 | 11.98 | 86.25 | 5.83 |
| Phenotypic variance | 94864.60 | 10.22 | 14.06 | 88.82 | 7.17 |
| PCV* | 17.01 | 22.75 | 30.92 | 32.95 | 8.62 |
| GCV** | 15.57 | 21.02 | 28.54 | 32.47 | 7.77 |
| ECV*** | 6.87 | 8.70 | 11.89 | 5.60 | 3.71 |
| Heritability | 83.71 | 85.37 | 85.22 | 97.11 | 81.41 |
| Genetic advance | 531.11 | 5.62 | 6.58 | 18.85 | 4.49 |
| Genetic gain | 29.34 | 40.01 | 54.27 | 65.91 | 14.45 |

*Phenotypic coefficient of variance, GCV** Genotypic coefficient of variance, ***Environmental Coefficient of Variance

**Table 3:** Biochemical analysis of different ecotypes of *Cordia myxa*

| Site | TSS* | Titrable Acidity | pH | Moisture content (%) | Total Sugar contents (%) | Ascorbic acid | Nitrogen (%) | Protein |
|---|---|---|---|---|---|---|---|---|
| 1.Suliali (Thana) | 5.167 | 1.690 | 6.42 | 90.67 | 1.517 | 28.59 | 1.530 | 11.41 |
| 2.Lappiana | 4.000 | 1.470 | 6.33 | 91.00 | 1.513 | 31.28 | 1.790 | 10.18 |
| 3. Raja Talab | 3.19 | 1.063 | 6.73 | 89.33 | 1.680 | 32.97 | 1.163 | 7.27 |
| 4. Shahpur | 4.003 | 0.793 | 6.66 | 77.33 | 1.733 | 30.37 | 1.050 | 6.56 |
| 5.Gangath | 4.633 | 1.387 | 6.57 | 81.05 | 1.633 | 25.77 | 1.357 | 8.47 |
| 6.Gumer | 5.00 | 1.427 | 6.57 | 87.63 | 1.270 | 26.99 | 1.630 | 9.53 |
| 7.Dehra | 5.000 | 1.330 | 6.70 | 80.70 | 1.993 | 36.72 | 1.153 | 6.99 |
| SE | 0.4701 | 0.043 | 0.12 | 0.954 | 0.103 | 2.1008 | 0.044 | 0.37 |
| CD $_{0.05\%}$ | 0.216 | 0.094 | 0.055 | 2.078 | 0.224 | 4.577 | 0.097 | 0.793 |

*Total Suspended Solids

**Figure 1**: Hierarchical cluster analysis using ward method (rescaled distance cluster combine)

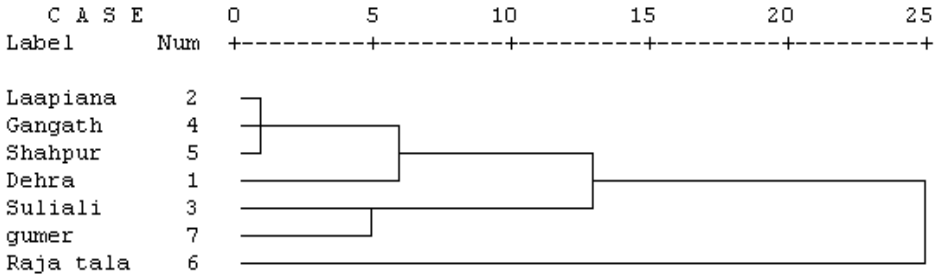


**Table 4**. Correlation among the fruit characters of different ecotypes of *Cordia myxa*

| Characters | Fruit weight | Seed Weight | Pulp weight | Seed Diameter | Fruit Diameter | Fruit Length |
|---|---|---|---|---|---|---|
| Fruit weight | 1 | -0.118 | 0.699 | -0.553 | 0.887** | 0.666 |
| Seed weight | | 1 | 0.238 | -0.529 | 0.165 | -0.601 |
| Pulp weight | | | 1 | -0.902** | 0.771* | 0.406 |
| Seed Diameter | | | | 1 | -0.732 | -.004 |
| Fruit diameter | | | | | 1 | .369 |
| Fruit length | | | | | | 1 |

## Conclusion

The main aim of any crop selection programme is to attain strains with higher yield and more adaptability under crop husbandry. As fruit traits and yield are complex trait the improvement can be attained only when genotypes are selected or improved for fruit and other contributing traits. For this purpose, information regarding coefficient of variation, heritability and genetic advance and biochemical traits of fruits is required. In the present study significant differences were observed among ecotypes for all the traits showing possibility of improvement of these. Low level of Environmental coefficients of variation than GCV and PCV among the selected traits strait suggest the reliability of selection of genotypes on the basis of phenotypes for improvement of species. Fruit weight, seed diameter and fruit diameters showed high heritability along with high genetic advance indicating that these characters were governed by additive gene effects and therefore, improvement can be achieved by direct selection. Hierarchical cluster analysis shows that the ecotypes are not so closely related and have variation among them. The Suliali and Lappiana ecotypes were found to best among all the ecotypes with better fruit size and nutritive value.

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