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Morphological, biochemical and molecular characterization of some promising potato (*Solanum tuberosum* L.) cultivars of Odisha

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Abstract
The main aim of this investigation is to assess the morphological, biochemical and molecular characterization of some important potato varieties of Odisha. Fifteen varieties of potato were characterized to find out their genetic diversity. The morphological and the biochemical parameters were also analysed and the tuber shape was found to be either oval or round. The tuber colour was almost brown or brownish yellow in all the cultivars except in Kufri Lalima which had brownish red type. The maximum tuber yield (20.24 t/ha) was obtained in Kufri Lalima followed by Kufri Khyati (19.11 t/ha). The maximum carbohydrate content was observed in variety Kufri Lauvkar (20.4 g/100g of fresh tuber) followed by Kufri Khyati (19.3 g/100g of fresh tubers) and the lowest carbohydrate content was found in Kufri Garima (15.11 gm/100g of fresh tubers). The highest amount of total chlorophyll was present in Kufri Khyati (4.151mg/g of leaves) followed by Kufri Himalini (3.326mg/g of leaves) and the lowest chlorophyll content was found in Kufri Shailja (1.268mg/g of leaves). The genetic diversity analysis using ISSR primers revelled that, maximum number of polymorphic bands was obtained using UBC810 primer. The average number of polymorphic bands was 4.66 per primer and the average percent polymorphism was found to be 91.66%. Polymorphism information content (PIC) was found to be the highest in UBC810 followed by UBC812. Average PIC content of primers among all 15 cultivers was found to be 0.568. Pair-wise estimates of similarity matrix ranged from 0.15 to 0.93 and average Jaccard's similarity calculated among all varieties was found to be 60.0%. Genetic distances obtained from the dendrogram could be helpful for the breeders to choose the diverse parents for a breeding program aimed at varietal improvement.

Keywords: Potato, ISSR marker, genotyping, chlorophyll content, carbohydrate content

Introduction
The global food price crisis experienced and the new recent rise in food prices are harbingers of these developments and have triggered serious concerns among a broader public and drawn renewed attention to the need for agricultural research. Further investments and advances in crop yields and productivity are important for raising the availability of food and preparing the global food system for the decades to come. Potato (*Solanum tuberosum* L.) is a crop of great importance and one of the four most-valuable crops worldwide, and exceeded only by wheat, rice and maize in world production for human consumption [1]. Thereby, potato can make an important contribution. For a number of reasons, potato stands out among the world's major food crops. Potato plays multiple and important roles in local food systems and for food security. It is well suited for cultivation in environmental conditions where other crops may fail and its short and flexible vegetative cycle makes it well suited for rotation with other major crops, such as wheat, rice, maize or soybeans. Thus, potato helps to increase the availability of food, contributing to a better land use ratio by raising the aggregate efficiency of agricultural production systems. By providing income generation opportunities as a cash crop and generating employment, potato contributes to alleviating poverty. Further, potatoes represent an important source of energy, with a high delivery of energy per unit land, water and time, and are a valuable source of minerals and vitamins for the diet [2]. Nutritive value of potato is relatively high, because of protein content and composition [3]. Potato is also characterised by high amounts of starch, and lower content of sugars, minerals and vitamins of B complexes, folic acid, fat-soluble vitamins E, K, and carotenoids, which may be converted into vitamin A [4]. The content of vitamins in tubers is not high; however, 200 g of potatoes covers much of the daily requirement for these compounds, especially vitamin B6 (20-26%), vitamin B1 (12-

20%), niacin (10-20%), folic acid (4-12%), and pantothenic acid (10%) [5]. Potato is an excellent source of vitamin C and other biologically active substances, such as polyphenols and flavonoids, which are commonly described as antioxidants [3, 4]. These substances have beneficial influence on human organism, as they protect against cardiovascular disease, and cancer, as well as reduce blood cholesterol level [6]. Potato is one of the most important vegetables consumed by the people of Odisha. It is being cultivated over 15000 ha with production 2.0-2.5 lakh million tones. The productivity of potato in Odisha is 16.48mt/ha. Here most of the potatoes are produced in Rabi season. Odisha produces about 20% of its own requirement of potato annually.

Major constrains of potato production in Odisha are completion for land, incidence of pests, limited supply of quality seed tubers in time, inadequate cold storage facilities, extension gap, low farm credit, inadequate irrigation and poor economic status of the farmers. Among the major crops, the potato probably has the largest number of wild relative species, and almost all wild species of potatoes are in the same gene pool, so there are many ways, to cross cultivated potatoes with them and exploitation of the useful properties. Development and utilization of genetic resources as well as germplasm conservation generally depends on a clear understanding of the genetic diversity and relationships between varieties from target regions. So, the present study was carried out at the Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha to perform the morphological, biochemical and molecular characterization of different genotypes of potato cultivated in Odisha to investigate their genetic diversity and biochemical characteristics so that these can be suitably used in future potato breeding and improvement programme.

Materials and Methods

Plant Material

Fifteen cultivars of potato were obtained from the All India Coordinated Project on Potato, Odisha University Agriculture and Technology (OUAT), Bhubaneswar, Odisha. These tubers were planted in the green house of Department of Agricultural Biotechnology, OUAT, Bhubaneswar for molecular and biochemical characterization. The different varieties of potato studied in the present investigation are (1) Kufri Ashoka (2) Kufri Shailja (3) Kufri Garima (4) Kufri Pushkar (5) Kufri Surya (6) Kufri Khyati (7) Kufri Himalini (8) Kufri Lauvkar (9) AICRP- P-23 (10) Kufi Gaurav (11) Kufri Lalit (12) Kufri Pukhraj (13) Kufri Lalima (14) Kufri Chipsona-3 (15) Kufri Jyoti.

Morphological characterization

Potato cultivars exhibited a wide variation in with respect to morphological features. The tuber colour, tuber shape, tuber flesh, tuber weight in gram (gm) and the yield (t/ha) were recorded using standard protocol.

Biochemical characterization

(i) Estimation of Chlorophyll content

Chlorophyll was extracted using 80% acetone and the absorption was measured at 663nm and 654nm wave length in a spectrophotometer. Using the absorption coefficient, the amount of chlorophyll present in the extract was calculated by:

mg chlorophyll a/ gm of fresh tissue= 12.7(A663)-2.69(A645) ×V/1000×W

mg chlorophyll b/gm of fresh tissue=20.2(A645)-4.68(A663) ×V/1000×W

mg total chlorophyll/gm of fresh tissue= 20.2(A645) +8.02(A663) ×V/1000×W

Where A= absorbance at the specific wavelengths

V= Final volume of chlorophyll extract in 80% acetone

W= Fresh weight of tissue extract

(ii) Estimation of total carbohydrate by Anthrone method

Fresh tuber samples (100 mg each) were taken in different boiling tubes. The boiling tubes were kept in a water bath for three hours with 5ml of 2.5N HCl for hydrolyses. Then it was kept in room temperature for cooling. After that the sample was neutralised with solid sodium carbonate until the effervescence ceases. Then the volume was made to 100ml and centrifuged. After that the supernatant was collected and from that 0.5ml of supernatant was taken for analysis. The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml from the working standard," 0" taken as blank. The volume was made to 1ml in all the tubes including the sample tube by adding distilled water. Then 4ml of anthrone reagent was added. The tubes were heated in a boiling water bath for eight minutes and were allowed to cool rapidly and the absorbance of green to dark green colour was taken at 630nm wave length. A standard graph was drawn by plotting concentration of standard on X- axis versus absorbance on the Y- axis. From the graph the amount of carbohydrate present in the sample was calculated by using the following formula.

$$\text{Amount of carbohydrate present in 100mg of sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Molecular characterization

(i) Genotyping of the potato cultivars with ISSR primer

The total genomic DNA was extracted from young leaf tissue of the potato genotypes [7]. The quality of DNA extracted was checked by electrophoresing the samples using 0.8 percent agarose gel and quantified using Nanodrop® ND-1000 Spectrophotometer. Polymerase chain reactions for ISSR analysis were carried out under standard conditions for all the primers using 1 U of Taq polymerase with 1X polymerase chain reaction buffer (100 mM Tris-HCl at pH 9, 500 mM KCl, and 15 mM MgCl2), 2.5mMdNTP, 3 mM $MgCl_2$, 20pM of each primer, and 50 ng of DNA template with a final reaction volume of 10µL. The PCR reactions were denatured at 94^0C for 5 minutes followed by 40 cycles of 94^0C for 1 minute, Ta (Table 1) for 1 minute and 72^0C for 1 minute. The final extension was 72 ^{0}C for 5 minutes. The amplified products were resolved in 2.5% agarose gel stained with ethidium bromide. Stained DNA/gels were placed on the Gel Doc. system (UVITECH, Cambridge, UK) and was photographed under U.V. light. Scoring of amplification product was done by '1' if allele is present and '0' when allele is absent.

Table 1: Primers used in the study

Sr. No	Primers	Sequence (5' ⟶ 3')	Annealing Temperature (Ta)
1	UBC810	$(GA)_8T$	55 °C
2	ISSR9	$(AC)_8YA$	57 °C
3	UBC812	$(GA)_8A$	55 °C

(ii) Similarity coefficient analysis

For similarity coefficient selected genotype was compared with the rest of genotypes. Greater the value of coefficient, compared variety will be more similar to selected variety. Jaccard's similarity coefficient was calculated [8] as follows;

$$\text{Jaccard's similarity Coefficient} = \frac{n_{xy}}{n_1 - n_2}$$

Where, n_{xy} = Number of bands common in sample a and b, n_1 = Total number of bands present in all samples and n_2 = Number of bands not present in sample a or b but found in other samples The similarity matrix was subjected to generate a dendrogram using software programme NTSYS pc Ver 2.1. Exeter Software, N.Y. [9].

(iii) Principle component analysis

The Jaccard's similarity matrix was subjected to principle component analysis (PCA). This coordination method makes use of multidimensional solution of the observed relationship. PCA resolves complex relationships into a function of fewer and simpler factor. In this, data matrix is derived from the distance or similarities between the operational taxonomic units.

(iv) Percent polymorphic Loci calculation

A locus is defined as polymorphic when the frequency of marker (allele) is <1.0. The percent of polymorphic loci was calculated by using the following formula.

$$\text{Percent polymorphic Loci} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands compared}} \times 100$$

(v) Polymorphic information content (PIC)

Most informative primers were selected based on the extent of polymorphism. The polymorphic information content (PIC) was calculated by applying the following formula [10].

$$PIC = 1 - \sum_{i=1}^{n} fi^2$$

where, fi^2 is the frequency of the i^{th} allele.

Result and Discussion

Morphological and Biochemical Characterization of potato cultivars

The biochemical observations revealed that, the maximum carbohydrate content (20.4 gm/100gm of fresh tubers) was observed in variety 'K.Lauvkar' followed by the variety (19.3 gm/100gm of fresh tubers) 'K.Khyati' and the lowest carbohydrate content was found in (15.11 gm/100gm of fresh tubers) 'K.Garima'. The highest amount of total chlorophyll was present in 'K.Khyati' (4.151mg/gm of leaves) followed by 'K.Himalini'(3.326mg/gm of leaves) and the lowest chlorophyll content was found in 'K.Shailja'(1.268mg/gm of leaves). Similarly, the carbohydrate content was estimated in potato cultivars [11]. In the similar way quantitative estimation of chlorophyll (Figure 1) and total sugar was done in potato cultivars and all these results were in relation with the work done in 2010 [12]. During the course of investigation, potato cultivars exhibited a wide variation in with respect to morphological features (Table 2). The tuber colour was almost same, either brown or brownish yellow except in K.Lalima it was brownish red type .The tuber shape was either oval or round (Figure 2). The tuber flesh was white creamy in all tubers except light yellow in case of K.Garima and K.Himalini. The yield data and tuber weight were taken after 75 days. The maximum potential yield was obtained in 'K.Lalima' (20.24 t/ha) followed by 'K.Khyati' (19.11 t/ha). The tuber weight was highest in 'K.Pushkar (37.58 gm). These obtained data could be helpful in the future potato improvement programme.

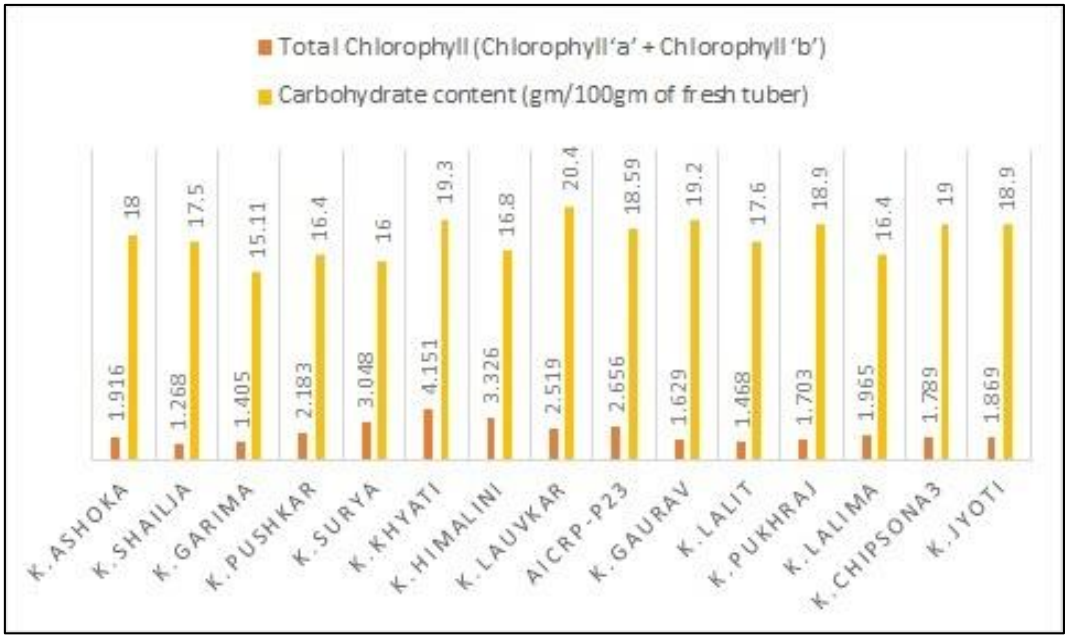

Fig 1: Chlorophyll content and Carbohydrate content in different potato cultivars

Fig 2: Potato cultivars exhibiting a wide variation with respect to morphological features

Table 2: Morphological characteristics of potato cultivars

Sl. No	Varieties	Tuber colour	Tuber shape	Tuber flesh	Tuber weight (g)	Yield (t/ha)
1	Kufri Ashoka	Brownish yellow	Oval	White creamy	27.94	16.09
2	Kufri Shailja	Brown	Oval	White creamy	23.11	9.81
3	Kufri Garima	Brownish yellow	Oval	Light yellow	28.56	11.41
4	Kufri Pushkar	Brown	Round	White creamy	37.58	14.99
5	Kufri Surya	Brownish yellow	Oval	White creamy	28.52	18.01
6	Kufri Khyati	Brown	Oval	White creamy	25.38	19.11
7	Kufri Himalini	Brownish yellow	Round	Light yellow	29.86	18.4
8	Kufri Lauvkar	Brownish yellow	Round	White creamy	29.11	12.3
9	Aicrp-P23	Brown	Oval	White creamy	25.23	14.11
10	Kufri Gaurav	Brown	Oval	White creamy	23.56	11.68
11	Kufri Lalit	Brown	Round	White creamy	26.99	18.08
12	Kufri Pukhraj	Brown	Oval	White creamy	21.19	16.39
13	Kufri Lalima	Brownish red	Round	White creamy	25.12	20.24
14	Kufri Chipsona3	Brown	Oval	White creamy	26.13	12.50
15	Kufri Jyoti	Brownish yellow	Oval	White creamy	27.19	17.34

Characterization of ISSR and data Analysis

Three Inter Simple Sequence Repeats (ISSR) primers were used to generate DNA fingerprint profile of 15 different cultivars of potato. The primer UBC810 (Figure 3B) produced the maximum 6 bands followed by 5 bands in UBC812 primer (Figure 3A). The lowest number of bands was observed in ISSR9 (4 bands) and the average number of bands per primer was found to be 5 (Figure 3C). The maximum number of polymorphic bands (6) was obtained using UBC810 primer. The average number of polymorphic bands was 4.66 per primer. The average percent polymorphism was 91.66%. Polymorphism information content was found to be the highest in UBC810 (0.743). Average PIC content of three ISSR primers among all 15 varieties was found to be 0.568 (Table 3). All the 15 potato cultivars used in the present investigation could be differentiated from one another on the basis of their complete ISSR profiles. These obtained data were in accordance to the following experiments conducted in potato as in potatoes, ISSRs have been successfully used for genomic fingerprinting [13], genetic diversity and phylogenetic assessment [14].

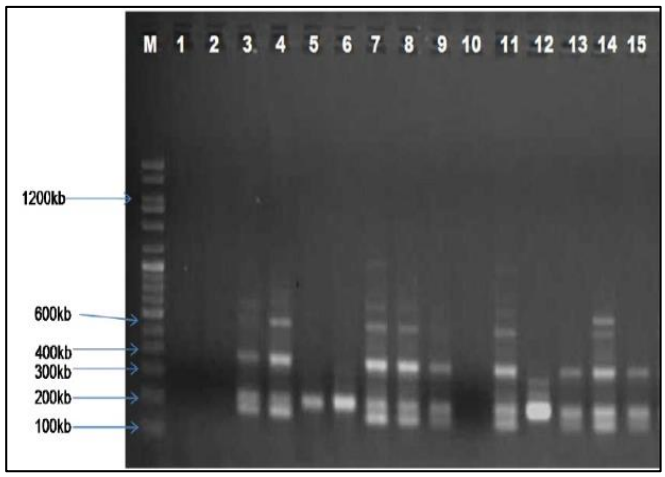

Fig 3A: DNA amplification profile generated in 15 cultivars of potato using UBC812 primer

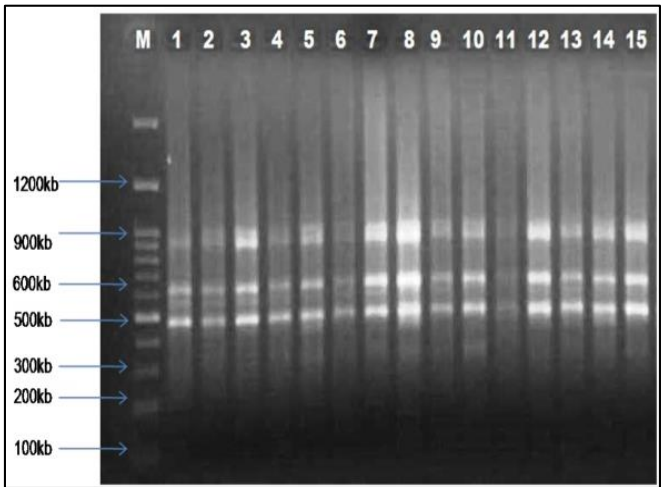

Fig 3B: DNA amplification profile generated in 15 cultivars of potato using UBC810 primer

Name on the top of the lane represents cultivars (1) K. Ashoka (2) K.Shailja (3) K. Garima (4) K. Pushkar (5) K. Surya (6) K. Khyati (7) K. Himalini (8) K. Lauvkar (9) AICRP P-23 (10) K. Gaurav (11) K. Lalit (12) K. Pukhraj (13) K. Lalima (14)K.Chipsona-3 (15)K.Jyoti

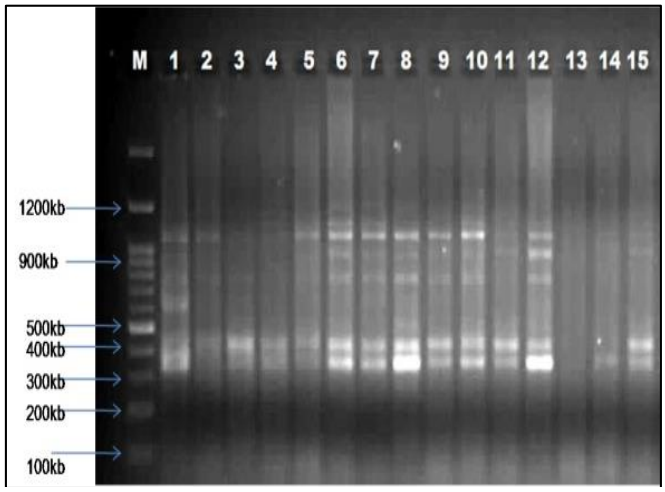

Fig 3C: DNA amplification profile generated in 15 cultivars of potato using ISSR 9 primer

Name on the top of the lane represents cultivars (1) K. Ashoka (2) K.Shailja (3) K. Garima (4) K. Pushkar (5) K. Surya (6) K. Khyati (7) K. Himalini (8) K. Lauvkar (9)AICRP P-23 (10) K. Gaurav (11) K. Lalit (12) K. Pukhraj (13) K. Lalima (14) K. Chipsona-3 (15) K. Jyoti

Table 3: DNA profile and polymorphism generated in 15 cultivars of potato using 3 ISSR primers

Primer Code	Total No. of bands (bp)	Monomorphic band (bp)	Polymophic band (bp)	%age Polymorphism (bp)	Size Range (bp)	Average PIC value
UBC810	6	0	6	100.0	350-1050	0.743
ISSR9	4	1	3	75.0	450-1000	0.248
UBC812	5	0	5	100.0	150-700	0.715
Total	15	1	14	275.0	-	1.706
Mean	5	0.33	4.66	91.66	-	0.568

Genetic diversity and relationships

Genetic relationships of 15 cultivars were determined on the basis of Jaccard's pair wise similarity coefficient. The DNA profile data derived from ISSR primers were subjected to calculate the genetic similarity and the similarity matrix was presented in Table 4. Pair-wise estimates of similarity matrix ranged from 0.15 to 0.93 and average Jaccard's similarity calculated among all cultivars was found to be 60.0%. The genotype pair K.Himalini and K.Lauvkar exhibited the highest similarity coefficient (0.93). The lowest similarity (0.15) was recorded between the variety pair K.Lalit and K.Shailja. Following data analysis based on Jaccard's similarity coefficient, a dendrogram was plotted for the data set (Figure 4). This revealed that, the cluster analysis by using UPGMA algorithm grouped 15 varieties into two major clusters I and II at 47% similarity coefficient. Cluster I comprised of 6 varieties namely K.Ashoka, K.Surya, K.Gaurav, K.Pukhraj, K.Khyati, K.Shailja. The varieties K.Garima, K.Pushkar, K.Jyati, K.Himalini, K.Lauvkar, AICRP P-23, K.Lalit, K.Chipsona-3, K.Lalima were placed in Cluster II. The varieties K.Himalini and K.Lauvkar were found to have the maximum Jaccard's similarity coefficient (93.0%).

Cluster I was further divided into three sub-clusters 'IA', 'IB' and "IC' at 51% similarity coefficient. Cluster II was further subdivided into four sub-cluster 'IIA','IIB', 'IIC', 'IID' at 55% similarity coefficient. Similar results were analysed [15], where 20 potatoes were identified in Czech Republic by using ISSR primers. Similarly, in 2015 evaluated 9 potato cultivars of Egypt on molecular basis using ISSR primers [16]. The data generated using three ISSR primers were used in PCA analysis using Jaccard's similarity coefficient (Figure 5, Figure 6). Principal Component Analysis (PCA), which is another representation of Jaccard's similarity coefficient, indicated a similar trend. The two-dimensional scaling of PCA analysis placed all the 15 cultivars of potato into four groups. Ist group contain K. Shailja, K. Ashoka, K. Khyati. IInd group contain K. Gaurav, K. Pukhraj, K. Surya, AICRP-P-23. IIIrd group contains K. Himalini, K. Lauvkar, K. Garima, K. Pushkar. IVth group contains K. Lalit, K.Lalima, K.Chipsona-3. Similar results were observed in 2015, where genetic diversity of 45 potato genotypes was assessed by using 5 ISSR primers [17]. ISSR marker superiority over other techniques, have been identified in several studies. Also, in another study, in 1999 concluded that 5 ISSR primers were adequate to distinguish 35 varieties of potatoes [18]. In 2009 also, thirteen ISSR primers were used to investigate the broad variability in cytoplasmic and nuclear DNA of *Solanum* sp. [19] genotypes regenerated plantlets and emphasized that ISSR markers, due to its fast, high reproducibility and low cost, according to obtained useful information, are suitable for the analysis of genetic variations in this method of proliferation.

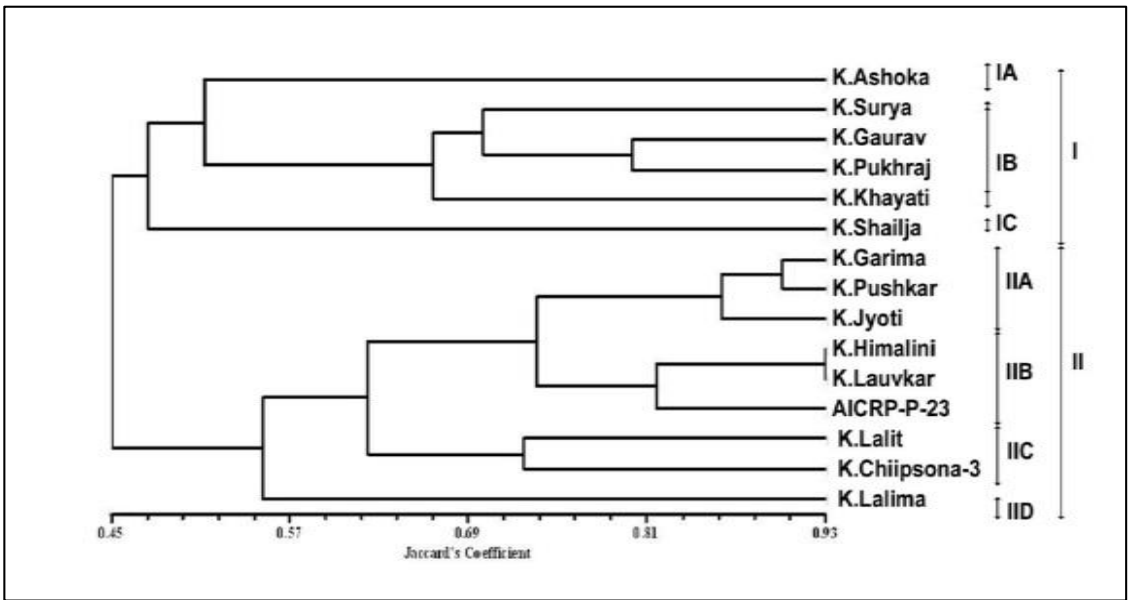

Fig 4: Dendrogram depicting genetic relationship among 15 cultivars of potato based on ISSR markers

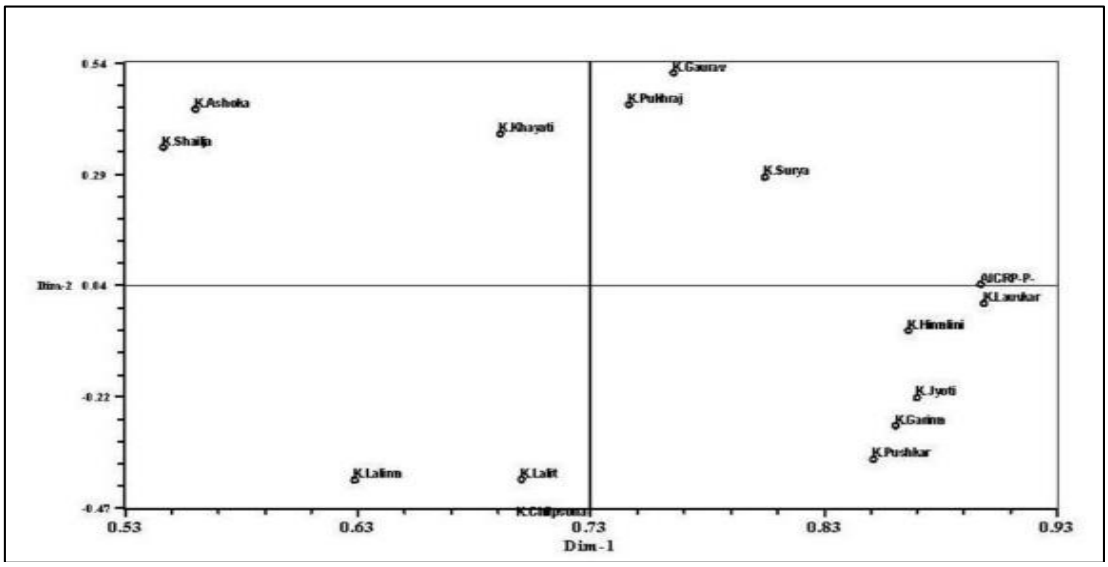

Fig 5: Two-dimensional scaling by principal component analysis (PCA) of 15 cultivars using Jaccard's similarity coefficient of ISSR profile data

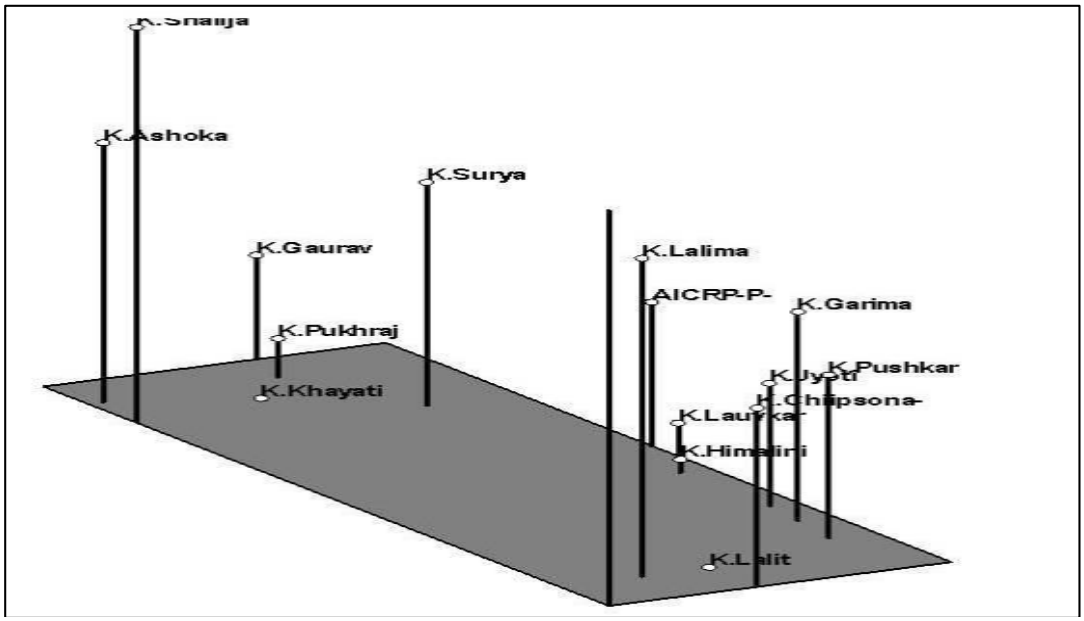

Fig 6: Three-dimensional scaling by principal component analysis (PCA) of 15 cultivars using Jaccard's similarity coefficient of ISSR profile data

Table 4: Similarity coefficient matrix of 15 cultivars of potato using 3 ISSR markers

Genotypes	K.Ashoka	K.Shailja	K.Garima	K.Pushkar	K.Surya	K.Khayati	K.Himalini	K.Lauvkar	AICRP-P-2	K.Gaurav	K.Lalit	K.Pukhraj	K.Lalima	K.Chiipson	K.Jyoti
K.Ashoka	1														
K.Shailja	0.5	1													
K.Garima	0.33	0.4	1												
K.Pushkar	0.31	0.36	0.9	1											
K.Surya	0.5	0.63	0.7	0.64	1										
K.Khayati	0.5	0.3	0.42	0.38	0.6	1									
K.Himalini	0.4	0.36	0.64	0.71	0.57	0.57	1								
K.Lauvkar	0.43	0.38	0.69	0.77	0.62	0.62	**0.93**	1							
AICRP-P-2	0.5	0.45	0.82	0.75	0.73	0.58	0.79	0.85	1						
K.Gaurav	0.6	0.56	0.5	0.46	0.7	0.7	0.64	0.69	0.67	1					
K.Lalit	0.21	**0.15**	0.58	0.67	0.38	0.5	0.71	0.64	0.5	0.36	1				
K.Pukhraj	0.45	0.4	0.5	0.46	0.7	0.7	0.64	0.69	0.67	0.8	0.36	1			
K.Lalima	0.3	0.38	0.67	0.6	0.4	0.27	0.43	0.46	0.55	0.25	0.45	0.25	1		
K.Chiipsona	0.33	0.27	0.64	0.73	0.42	0.31	0.64	0.57	0.54	0.29	0.73	0.29	0.67	1	
K.Jyoti	0.31	0.36	0.9	0.82	0.64	0.5	0.71	0.77	0.75	0.58	0.67	0.58	0.6	0.58	1

Conclusion

Morphological, biochemical and molecular analysis makes it possible to establish differences at various taxonomic levels which in turns help the researchers to assess genetic diversity in the investigated germplasm of potato. In the present study, the morphological parameters were employed to assess the difference between the genotypes of potatoes. It will be helpful for the researchers for better understanding of selection of genotypes for potato improvement programmes. The biochemical assessment concluded that, the maximum carbohydrate content was observed in variety 'K. Lauvkar' followed by the variety 'K. Khyati' and the lowest carbohydrate content was found in 'K. Garima'. The highest amount of total chlorophyll was present in 'K. Khyati' followed by 'K. Himalini' and the lowest chlorophyll content was found in 'K. Shailja'. These could be helpful for the researcher to select the prominent genotypes in future potato improvement programmes. The DNA profiling and the cluster analysis of the genotypes grouped 15 cultivars into two major clusters I and II at 47% similarity coefficient. The cultivars K. Himalini and K. Lauvkar were found to have the maximum Jaccard's similarity coefficient. The cluster analysis could be helpful in plant breeding to make decision regarding the selection of diverse parents from inter clusters for breeding programs in order to maximize the expression of heterosis for any desired character of agronomic importance. ISSR markers were successfully used for the quick identification of varieties, to investigate diversity between different varieties of potato. Although potatoes have a narrow genetic base because of its propagation, in this study the molecular characterization of microsatellite showed a significant genetic variability and high levels of polymorphism. The present study revealed the existence of high level of genetic diversity among the 15 cultivars of potato. These varieties can further be used as parental material for fixation of heterosis in potato improvement program.

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Conflict of interest

The authors declare that they have no conflict of interest.

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