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Heredity divergence and genetic components of local potato (*Solanum tuberosum* L.) cultivars as revealed by molecular markers and quantitative traits

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Abstract

Molecular marker association with morphological traits have crucial role for heredity enhancement of local cultivars in different crops. In the present investigation, local potato cultivars were characterized and evaluated using SSRs markers and quantitative traits to recover the heredity divergence and the genetic components were estimated. All seven SSRs were showed 100% polymorphism with 3.71 allele per locus in the study. Based on different marker efficiency parameters, SSR0707 and STI0012 were most efficient primer to distinguish the local potato cultivars. The results of allelic diversity, genetic differentiation and AMOVA indicated that the genetic variability is greater within the population of potato cultivars. All the genotypes were grouped into three cluster as revealed by the Dendrogram utilizing SSRs. Different genetic components and principal component analysis using nine morphological traits suggested that average tuber weight, average tuber length, number of tubers per plant and plant height are potential traits for early generation selection by breeder to develop high tuber yielding potato. Total two phenotypic groups were found based on average silhouette method using nine quantitative traits in the current population. The knowledge acquired from this study, will be helpful for genetic improvement of local potato cultivars by plant breeders.

Keywords: Potato (*Solanum tuberosum* L.), Simple Sequence Repeats (SSR), quantitative traits, genetic components and average silhouette method

Introduction

Potato (Solanum tuberosum L.) is the top fourth food crop after maize, rice and wheat (Zeng et al., 2020)^[56]. India is the second country after China to produce large quantities of potatoes *i.e* 12% of global production and Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal, Gujarat and Madhya Pradesh are major contributing states (Reddy et al., 2018)^[45]. India is one of the priority areas for investment in potato research and innovation (Devaux et al. 2014) [13]. According to the Global Hunger Index (GHI), forty seven countries still have 'serious' and 'alarming', and one in 'critically alarming' hunger levels (IFPRI 2019)^[29]. So, the availability of nutritious food brings down the hunger value and therefore GHI as well. Potato is a wholesome and versatile food with more favourable overall nutrient-to-price ratio than most of the fruits and vegetables (Drewnowski and Rhem, 2013) ^[15]. Furthermore, potatoes produce more food per unit of water and cropland in less time than any other major crop and are up to seven times more efficient in using water than grains (FAO 2009 & NPC 2016)^[19,41]. Potato is grown as a staple food in agriculture-based countries like India, and as a high-value crop in urbanized and transforming countries. Thus improvement of potato plays a key role in improving global food security and reducing poverty (Devaux et al. 2014) [13]. Little or no efforts have been made to characterize local cultivars of potato in the state West Bengal of India. Modernization bottleneck leads to genetic erosion (Louwaars 2018) [34], generally by ignorance towards landraces and local cultivars. Collection, characterization, and classification of germplasm with morphological and molecular markers are crucial for any crop improvement (Govindaraj 2014) [26]. So identification of genetic diversity through proper investigation of local potato cultivars and subsequent utilization of useful cultivars in crop improvement is imperative (Ferguson 2007)^[21]. Genetic relatedness of potato genotypes was most extensively investigated through naked eye polymorphism (Kolech et al., 2016; Mishra et al. 2017; Anouma et al. 2017; Nasiruddin et al. 2017; Berdugo-Cely et al. 2017; Prabha et

al. 2018) [33, 37, 7, 38, 9, 44] and DNA markers like SSRs (Bali et al. 2018; Wang et al, 2019; Cruz et al. 2020) [8, 52, 11], EST-SSR (Salimi et al. 2016) ^[46], inter-simple sequence repeats (ISSRs) (Mahgoub et al. 2014) [35] AFLP (Wang et al., 2013) ^[51], SNPs (Kolech. 2016; Berdugo-Cely et al. 2017) ^[33, 9] and RAPD (Hoque et al. 2013)^[28]. Among them SSRs being the most common as they are co-dominant, randomly distributed throughout the genome, highly polymorphic, high reproducibility, less cost of operation, hyper-variability, amenable to automation, ease of multiplexing, highly conserved across related species and use with low quality DNA (Ghislain et al. 2009; Mason 2015; Yang et al. 2015; Jian et al. 2017; Duan et al. 2018) ^[23, 54, 31, 16]. To explore the extensive knowledge of heredity structure and yield assessment, consolidation of phenotypic and SSR markers have been established their readiness in rice (Ahmad et al., 2015) ^[5]. Consequently, the present investigation was carried out to obtain the genetic heterogeneity using SSRs marker and quantitative traits in local potato cultivars. In addition, the genetic components of quantitative traits were also estimated to sort out the potential cultivars for future breeding programme for improvement of local potato.

Materials and Method

Plant materials and design of experiments

Fourteen potato cultivars including ten local potato clones and four released varieties were considered in this study. The genotypes were collected from northern part of West Bengal, India by Central Germplasm Conservation Unit, Directorate of Research, Uttar Banga Krishi Viswavidyalaya. The present investigation was conducted at Agricultural Research Farm, Regional Research station, Terai zone, Pundibari, Cooch Behar, West Bengal during rabi season of 2017-18 and 2018-19. Complete randomized block design (CRBD) with three replications was adopted to successfully carry out the experiment.

Morphological data collection

The quantitative value of eight morphological characters *viz.* sprout length (SL), plant height (PH), leaf length (LL), leaf width (LW), number of tubers per plant (NT), tuber length (TL), tuber weight (TW) and tuber yield per plant (TY) were recorded on five randomly selected plants, excluding the plants located at borders to avoid border effect so that highest precision could be achieved. The germination percentage of potato tubers was calculated as described by Clarke and Stevenson, 1943 ^[10]. All the data were recorded from three replication of the experimental plot and their average value of each replication were used for statistical analysis.

DNA isolation and PCR amplification

DNA was extracted from 80 mg fresh leaf samples of 14 potato genotypes following the method of Mandal *et al.*, 2016 ^[36]. The extracted DNA samples were stored at -20°C until use. The quality of extracted DNA was analyzed by 0.8%

agarose gel electrophoresis in TBE buffer, followed by ethidium bromide staining and visualized in gel documentation system. The purity of the DNA was estimated by spectrophotometry observing A260nm/A280nm ratio, and the yield was estimated by measuring absorbance at 260 nm. Seven SSR primer sets distributed on seven chromosome developed by Ghislain et al., 2004; Ghislain et al., 2009 [24]; Xiaoyan et al., 2016 [49] were used for this study and were synthesized by Sigma-Aldrich, India. PCR amplification was carried out in a volume of 25 µl containing 2 µl of 10 ng/µl DNA, 1 µl of each primer (100 ng/ µl), 1 µl of 2.5 mM dNTPs, 2.5 µl of 10X reaction buffer (Thermo Fisher Scientific, India), 0.2 µl of 5 U/µl Taq polymerase (Thermo Fisher Scientific, India) and 16.8 µl HPLC grade water. PCR reactions were performed in Veriti Thermal Cycler (Applied Biosystems, USA), an initial step for 5 min at 94°C with 35 cycles for 1 min at 94°C, 45 s at 52-58°C and 1 min at 72°C, and a final step of 5 min at 72°C. The PCR products were separated on 1.5% agarose gel with ethidium bromide staining and visualized in UV gel documentation system.

Data analysis

Marker efficiency parameters for SSRs viz. polymorphic information content (PIC), marker index (MI), resolving power (RP), discriminating power (D_i) and effective number of patterns per assay unit (Pt) were calculated using Microsoft Excel as described by Zargar et al., 2016 and Mandal et al., 2016^[36]. Allelic diversity and analysis of molecular variance were performed with the help of Gen AIEx 6.5 software (Peakall and Smouse, 2012). Genetic differentiation (G_{ST}) and gene flow (Nm) were estimated utilizing Pop Gen v 1.32 software (Yeh, 1997). Cluster analyses were implemented by UPGMA method, and the corresponding dendrogram was constructed using DARwin v 6.0 software. In order to estimate the goodness of fit between similarity matrix and the dendrogram, the coefficient of cophenetic correlation was calculated with help of R v 3.6.0 software with "dendextend" package. Phenotypic data analysis were implemented through R v 3.6.0 software using "Agricolae" package.

Results

Polymorphism and marker efficiency

A total 26 number of allele revealed by seven pairs of SSR markers, which were used for molecular characterization of 14 potato cultivars in the present investigation. The amplified product size ranged from 60 bp (STI0032) to 250 bp (STM2022) with an average number of allele per marker 3.71. In the current study, all the marker showed 100% polymorphism and no rare allele (less than 5% among the cultivars) was observed. To estimate the performance of SSRs markers different efficiency parameters *viz.* polymorphic information content (PIC), resolving power (RP), marker index (MI), discriminating power (D_j) and effective number of patterns per assay unit (P_t) were calculated in this investigation (Table 1).

Table 1: Estimation of different marker efficiency parameters reveled from SSRs profiling

Marker Id	Allele size (bp) range	NTA	NPA	NTB	PIC	RP	MI	Dj	Pt
STG0016	130-180	4	4	18	0.20	1.14	3.67	0.24	1.26
STM2022	190-250	5	5	17	0.39	1.57	6.68	0.51	1.65
SSR0707	130-180	3	3	14	0.56	2.00	7.86	0.60	2.28
STI0012	170-190	3	3	13	0.61	1.57	7.89	0.65	2.55
STI0032	60-140	3	3	15	0.48	1.57	7.27	0.53	1.94

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PM0938 120-160 3 3 19 0.12 1.29 2.23 0.15 1.13 Mean 3.71 ± 0.36 3.71 ± 0.36 16.86 ± 1.18 0.35 ± 0.08 1.55 ± 0.11 5.37 ± 1.00 0.40 ± 0.08 1.70 ± 0.20	SSR0675	90-180	5	5	22	0.09	1.71	2.02	0.14	1.10
Mean 3.71 ± 0.36 3.71 ± 0.36 16.86 ± 1.18 0.35 ± 0.08 1.55 ± 0.11 5.37 ± 1.00 0.40 ± 0.08 1.70 ± 0.20	PM0938	120-160	3	3	19	0.12		9.93		1.13
	Mean		3.71 ± 0.36	3.71 ± 0.36	16.86 ± 1.18	0.35 ± 0.08	1.55 ± 0.11	5.37 ± 1.00	0.40 ± 0.08	1.70 ± 0.22

[NTA = Number of total Allele; NPA = Number of polymorphic Allele; NTB = Number of total bands; PIC = Polymorphic information content; RP = Resolving power; MI = Marker Index; D_j = Discriminating power; Pt = effective number of patterns per assay unit]

The PIC values varied from 0.09 (SSR0675) to 0.61 (STI0012) with an average 0.35 per locus. The estimates of resolving power were found to be the highest for the marker SSR0707 (2.00), followed by SSR0675 (1.71) and was lowest for the marker STG0016 (1.14). The average MI value was 5.37 per SSR and ranged from 2.02 for SSR0675 to 7.89 for STI0012. The highest D_j was found for STI0012 (0.65) and lowest was for SSR0675 (0.14) with an average value of 0.40. The maximum number of P_t were observed for STI0012 and minimum number were for SSR0675 with the average value 1.70 which informed that the individual SSR markers have the ability to acclaimed almost 2 accessions within the infinite population size of potato. Association analysis showed that all

the efficiency parameters have positive significant correlation with each other with the except of resolving power.

Allelic diversity, AMOVA and gene differentiation

Allelic diversity was obtained from the two groups of potato accessions using the average number of different allele (N_a) , average number of effective allele (N_e) , Nei's gene diversity (h) and Shannon's information index (I). Group 1 which included local potato cultivars showed maximum N_a and highest value for I whereas group 2 showed the maximum N_e , greater value for h and uh. N_a , N_e , I, h and uh value for overall total potato collection were 1.48, 1.47, 0.40, 0.27 and 0.33 respectively.

PopulationNNaNeIhuh									
10.000	1.654±0.15	1.423±0.06	0.407±0.05	0.265±0.03	0.295±0.03				
4.000	1.308±0.18	1.508±0.09	0.391±0.06	0.274±0.04	0.365±0.06				
Total 7.000 1.481±0.12 1.465±0.05 0.399±0.04 0.270±0.03 0.330±0.03									
2	4.000 7.000	4.000 1.308±0.18	4.000 1.308±0.18 1.508±0.09 7.000 1.481±0.12 1.465±0.05	4.000 1.308±0.18 1.508±0.09 0.391±0.06 7.000 1.481±0.12 1.465±0.05 0.399±0.04	4.000 1.308±0.18 1.508±0.09 0.391±0.06 0.274±0.04				

 $[N = Number of potato clones; N_a = Number of different Alleles; N_e = Number of effective Alleles; I = Shannon's information index; h = Nei's gene diversity; uh = Unbiased Nei's gene diversity]$

The results of analysis of molecular variance (AMOVA) showed that 93% variation within the population and 7% variation among the population (groups). The gene

differentiation value (G_{ST}) and gene flow (N_{m}) between the two potato groups were 0.1241 and 3.5283 respectively.

Table 3: Analysis of molecular variance among the two groups of potato revealed by SSRs markers

Source	df	SS	MS	Est. Var.	%	FST	P(rand >= data)
Among Pops	1	5.679	5.679	0.283	7%		
Within Pops	12	48.750	4.063	4.063	93%		
Total	13	54.429		4.345	100%	0.06	0.183

Cluster analysis based on SSRs

All the fourteen potato cultivars were grouped into three cluster at the similarity coefficient of 0.65 as the result of dendrogram analysis based on UPGMA method (Figure 1). Cluster I had nine number of genotypes followed by cluster

III which included three number of genotypes and cluster II containing only two genotypes. The range of genetic similarity was observed between the cultivars from 46% (Kufri Arun and LPCH-01; LPCK-01 and K. Poukhraj) to 100% (LPCD-01 and LPCD-02).

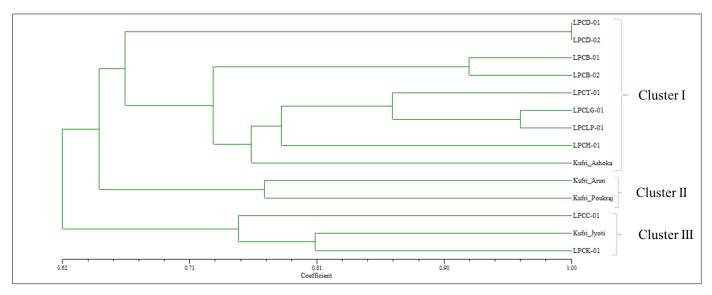


Fig 1: A dendrogram accomplished using 26 allele of seven SSRs

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Phenotypic variability

Nine morpho-phenetic quantitative characters *viz.* germination percentage (GP), sprout length (SL), plant height (PH), leaf length (LL), leaf width (LW), number of tubers per plant (NT), average tuber length (TL), average tuber weight (TW) and yield per plant (TY) were evaluated in two successive 'rabi' season on 14 potato cultivars. The result of ANOVA explained that all the cultivars had significant variation (p< 0.01) based on the morpho-phenatic characters. The cultivars LPCD-01 showed the shortest sprout length (1.00 cm), lowest plant height (15.38 cm), minimum leaf length (10.26 cm), smallest tuber length (1.78 cm) and lower value for tuber weight (1.59 g). Minimum germination percentage (92%), lowest leaf width (5.81 cm) and maximum average number of tubers per plant (40.82) were found in the genotypes LPCD-02. The cultivar LPCLP-01 had the prolonged sprout length (1.82 cm), least average number of tubers per plant (2.88) and lowest tuber yield per plant (394.50 g). Longest plant height (42.40 cm), highest average tuber weight (6.05 g) and maximum tuber yield (3080.83 g) were obtained by the cultivars Kufri Arun.

Table 4: ANOVA represented mean square value of r	nine quantitative traits
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		Sources of variance							
Characters	Mean sum of squares								
	Rep	Genotype	Year	Genotype × Year	Residual				
Degrees of freedom (DF)	2	13	1	13	54				
Germination percentage (GP)	0.16	34.61**	5.25	0.28	5.29	98.06			
Sprout length (SL)	0.00	0.32**	0.00	0.01	0.05	1.43			
Plant height (PH)	6.30	312.44**	0.96	0.48	4.56	23.73			
Leaf length (LL)	6.98	87.01**	0.06	0.15	1.18	16.04			
Leaf width (LW)	6.51	57.66**	0.12	0.07	0.54	11.01			
Number of tubers per plant (NT)	4.09	977.08**	2.40	0.72	5.40	10.10			
Average tube length (TL)	0.42	23.53**	0.05	0.01	0.16	5.61			
Average tuber weight (TW)	0.19	9.42**	0.50	0.02	0.09	4.22			
Tuber yield per plant (TY)	27816.00	155134.00**	7180.00	1425.00	4007.00	245.20			

Genetic components and association among the nine quantitative traits

In the present study, GCV and PCV ranged from 2.07% (GP) to 125.88% (NT) and from 3.07% (GP) to 127.19% (NT) respectively. Among the nine traits, PH, LL, LW, NT, TL, TW and TY were showed the higher percentage (> 20%) of

GCV and PCV whereas, displayed moderate GCV and PCV value. The broad sense heritability varied from 45% (GP) to 98% (LW, NT, and TL) for nine morpho-phenetic traits. NT (256.63%) showed the uppermost value for genetic advance followed by TY (125.29%) whereas, GP have lowest value (2.87%).

Characters	GCV (%)	PCV (%)	ECV (%)	H ² bs	GAM (%)
Germination percentage (GP)	2.07	3.07	2.27	0.45	2.87
Sprout length (SL)	14.17	19.95	14.05	0.50	20.73
Plant height (PH)	30.04	31.13	8.14	0.93	59.74
Leaf length (LL)	23.49	24.23	5.95	0.94	46.91
Leaf width (LW)	28.04	28.38	4.37	0.98	57.07
Number of tubers per plant (NT)	128.88	127.19	18.25	0.98	256.63
Average tube length (TL)	35.15	35.57	5.47	0.98	71.54
Average tuber weight (TW)	29.51	29.92	4.95	0.97	59.96
Tuber yield per plant (TY)	64.32	68.01	22.12	0.89	125.29

 Table 5: Predicted genetic components for different quantitative traits in potato

The result of genotypic and phenotypic correlation explained that, PH, LL, LW, TL and TW had the highly significant (p< 0.01) positive correlation with TY. NT showed the significant (p< 0.05) negative correlation with TY at genotypic level but phenotypically the relationship was non-significant.

Principal Component analysis

First three PCs with Eigen value greater than one acquired 92.41% of total variation as revealed from nine morphological traits. TW, TL, NT, LW and LL in the PC1, GP, TY, PH, SL and NT in the PC2, and PH, LW, LL and SL in the PC3 had individually more than 10% contribution towards the variability. According to the PCA-biplot, all the fourteen

cultivars were grouped into two distinct cluster. Cluster I had the two genotypes and rest of the genotypes were placed into the cluster II.

Genetic structure and dendrogram analysis

The average silhouette width illustrated a recognizable peak at 2 (K = 2) which predicted that, the 14 potato cultivars were classified into the two phenotypic groups (Figure 2) with nine morpho-phenetic traits. A dendrogram was constructed based on mean phenotypic performance with 14 potato cultivars using Euclidian distance method (Figure 3).

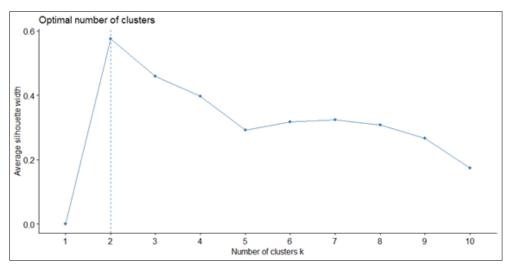


Fig 2: Estimation of optimum number of cluster using average silhouette method

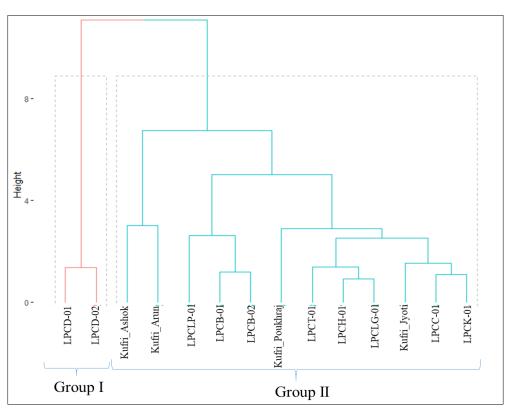


Fig 3: Dendrogram constructed from quantitative traits using Euclidian distance

Discussion

Estimation of genetic variability with molecular marker and morpho-phenentic traits together gives the in depth knowledge about the genetic structure and heredity information of local germplasm (Ahmad *et al.*, 2015 and da Silva *et al.*, 2017) ^[5, 12]. In the present study, seven pairs of SSRs markers which were distributed in seven number of chromosome and nine quantitative traits were used for the evaluation of 14 local potato cultivars. The amplified allele size produced by the SSR marker in 14 potato cultivars was minimum for STI0032 (60 bp) and maximum for STM2022 (250 bp). The results agreed with previous findings where the product size ranged from 106 bp (STI0032) to 244 bp (STM2022) reported by Xiaoyan *et al.*, 2016 ^[49]. Average allele per SSR found in this investigation was 3.71 which was relatively less from the earlier study by Xiaoyan *et al.*, 2016

^[49] (8.2 per SSRs) and Ghebreslassie et al., 2016 (8.0 per SSRs. According to DeWoody et al., 1995, A SSRs marker will be informative if their PIC value is more than 0.50. Based on this report, SSR0707 (0.56) and STI0012 (0.61) were the most significant SSRs markers for evaluation local potato cultivars in this investigation. In addition, STI0012 showed highest value for marker index, discriminating power and effective number of patterns per assay unit but moderate value for resolving power. The relationship among the different marker efficiency parameters suggested that all the parameters more or less equally important except resolving power. Hence, considering PIC, MI, D_i, and P_t most efficient SSRs were STI0012 followed by SSR0707 for evaluation of local potato cultivars under this study. For example, Xiaoyan et al., 2016^[49] also found that STI0032, STI0012, SSR0707 and STG0016 were most informative SSR marker for molecular dissection of 192 potato cultivars.

The fourteen potato clones were classified into two groups' viz. group I that included 10 local potato cultivars from northern part of West Bengal and group II with 4 released varieties from the CPRI, Shimla, India to estimate the allelic diversity and to acquire the satisfactory knowledge of gen diversity. Among the 26 number of allele reveled by seven SRRs markers, 22 were found in group I and 18 were found in group II with 14 number of common allele. Group I have higher number of different allele and lower number of effective allele as compared to group II. The Shannon information index (I) was greater for the group I which indicated higher allelic richness and homogeneous scattering of the allele within the local potato collections. Group II population showed the higher level of gene diversity in comparison with group I which explained that the released varieties have the broad genetic base. The genetic variability within population was significantly higher than the variation between the group I and group II potato collections as demonstrated by AMOVA. The genetic differentiation value also suggested that more numbers of identical potato clones are present in the current population. The gene flow between the local potato cultivars and released varieties was higher means evolutionary the genotypes of these two groups have close ancestry and narrow genetic diversity between the populations. Allelic diversity, genetic differentiation and AMOVA were also performed for exploring the genetic variability by Juyó et al., 2015 [32] with Phureja potato collection using SSRs markers.

The dendrogram constructed by the genotyping data revealed from seven SSRs markers showed high range of genetic relatedness and formed three cluster. Cluster I consisted with eight number of local potato cultivars viz. LPCD-01, LPCD-02, LPCB-01, LPCB-02, LPCT-01, LPCLG-01, LPCLP-01 and LPCH-01 and one released variety (Kufri Ashok). Reaming three potato released varieties were placed in to the cluster II and cluster III whereas rest of the two local potato cultivar were situated in the cluster III. Among the potato clones of cluster I, LPCD-01 and LPCD-02 exhibited 100% similarity, LPCLG-01 and LPCH-01 showed 96.15% similarity and LPCB-01 and LPCB-02 demonstrated 92.31% genetic identity. The estimated cophenatic coefficient value of dendrogram was 0.80 which indicated that the excellent ability of genotypic data revealed from the SSRs profiling for genetic variability analysis (Ahmad et al., 2015)^[5].

Fourteen potato cultivars were also evaluated based on their nine quantitative characters recorded in successive years. Analysis of variance explained that all the traits have significant (p< 0.01) variation among the cultivar at genotypic level. The interaction between genotype and year was nonsignificant may be due to the consistent phenotypic performance of genotypes for all the traits in two subsequent years. An expanding range (2.88 to 40.82) of number of tuber per plant was found in local potato cultivars with an average

value 11.94 whereas the average number tuber per plant was 5.50 in release varieties. The average tuber length and tuber weight indicated that local cultivars (TL = 5.05 cm; TW = 3.87 g) have small size tuber in comparison with release varieties (TL = 7.02 cm; TW = 5.12 g). The yield performance of the release varieties (2059 g) was significantly (p < 0.05) higher than the local potato cultivars (892.92 g). In spite of low yield, farmers are more prefer the local potato cultivars than the released varieties due to some qualitative traits like colour (anthocyanin content), taste preference (total solid and starch content), disease resistance and long term storage capability. For instance, plant height, leaf area, number of tubers per plant, average tuber length and tuber yield showed significant variation among the potato accessions as previously reported by Nasiruddin et al., 2017 and Fekadu et al., 2013 [38, 20].

Among the 14 potato cultivars, number of tuber per plants showed the highest GCV and PCV with moderate ECV, suggestive that selection could be effective with these traits in future breeding programme. Yield have second highest GCV and PCV but showed the higher ECV value means the greater environment as influence on this may be misleading for selection by breeders. High H^2_{bs} with high GAM (%) were observed for the plant height, leaf length, leaf width, number of tubers per plant, tuber length, tuber weight and tuber yield which were inhibited by the additive gene action and will be beneficial for early generation selection by potato breeders (Gopal., 199; Adjah *et al.*, 2020)^[25, 1].

Among the nine quantitative traits, plant height, leaf length, leaf width, tuber length and tuber weight showed significant positive relationships with tuber yield per plant and the higher degree of genotypic correlation coefficient value than the analogous phenotypic correlation suggested that selection made through these traits will be rewarding for development of high yielding genotypes. Identical findings in potato also made known by Nasiruddin *et al.*, 2017 ^[39] and Fekadu *et al.*, 2013 ^[20].

PCA have the important role to select the morphological traits for breeding programme in different crops including potato (Heberger et al. 2003; Placide et al. 2015; Yuan et al., 2016) ^[27, 43, 55]. The results of PCA described, average tuber weight, average tuber length, number of tubers per plant, leaf length, leaf width, tuber yield per plant and plant height had the major contribution towards genetic variability in potato genotypes. These findings indicated that, genetic enhancement possible in local potato cultivars if selection was made through these phenotypic characters. This finding also is in agreement with genetic component analysis. PCA provided the necessary knowledge for development of high yielding potato genotype through selection of suitable morphological traits like tuber size (Yuan et al., 2016 and Struik and Wiersema 1999) [55, 50]. Three cluster were found among the 14 potato genotypes with common phenotypic performance revealed by PCA-biplot (Figure 4).

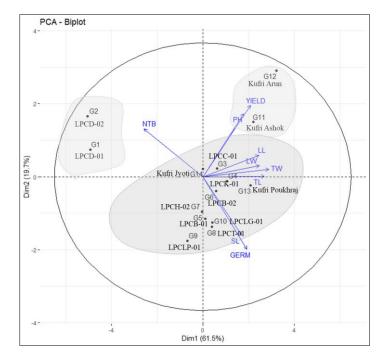


Fig 4: PCA-biplot revealed from the nine quantitative traits of 14 potato cultivars

Cluster I had two cultivars *viz*. LPCD-01 and LPCD-02 which are 100% genetically similar to each other based on SSRs profiling and produced higher number of tubers per plant. Cluster II consisted also two release varieties namely Kufri Ashok and Kufri Arun with high tuber yield potentiality. Cluster III contained ten genotypes with moderate phenotypic performance. This finding illustrated that hybridization between the genotypes of cluster I and cluster II may produce high yielding genotypes with greater number of tuber per plant. Cluster analysis based on PCA biplot generated through morphological traits were reported in pea (Espósito *et al.*, 2007) ^[18] and sweet potato (Afuape, 2011 and Afuape *et al.*, 2015) ^[2, 4].

The average silhouette width method presumed and validated

the number of rational cluster generated from morphological traits (Peter, 1987) ^[42]. This method divided the fourteen potato genotypes into two phenotypic groups (PG) *viz*. PG I included only two genotypes with small tuber size and greater number tubers per plant and PG II had twelve genotypes which have normal tuber size and higher tuber yield (Figure 5). This method was also used to find out the phenotypic population group in blackberry described by Jared *et al.*, 2019 ^[30]. Dendrogram obtained from the morphological data based on the Euclidian distance generated two cluster same as the average silhouette width method. The cophenatic coefficient value revealed from dendrogram was 0.99 (> 0.80) indicated that best fit of phenotypic data for analysis of diversity in potato.

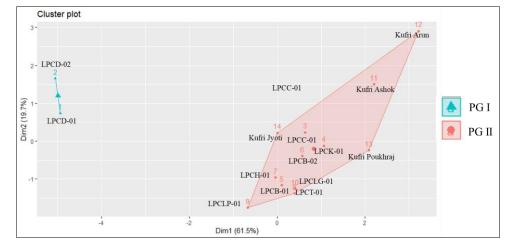


Fig 5: Cluster plot generated from nine quantitative traits using average silhouette method

Conclusion

Overall findings suggested that adequate genetic variability exist in the local potato cultivars taken into consideration in this study based on genotyping and phenotyping. Both SSRs and morphological markers together scrupulously established their competency to scrutinize the heredity information in potato cultivars. SSRs markers also secured that genetic diversity was higher in released varieties whereas greater allelic richness and Shannon information index were higher in local potato collections. Genetic components analysis and PCA based on the quantitative traits illustrated that synchronous choice of tuber size (average tuber weight and average tuber length), number of tubers per plant, leaf size (leaf length and leaf width) and plant height might be impressive in enhancement of tuber yield in local potato. Finally cluster study concluded that heredity intensification could be possible though hybridization programme between small sized potato cultivars (LPCD-01 and LPCD-02) and released varieties (Kufri Ashok and Kufri Arun).

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