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RESEARCH ARTICLE

Characterization of traditional small potato (*Solanum tuberosum* L.) cultivars for nutritional, quality traits and ploidy level

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Abstract

Traditional small potato cultivars of North Eastern region of India are an important food source, but no documentation is available regarding their ploidy level and nutritional worth. The study was undertaken to explore these cultivars to determine their ploidy level by flow cytometry method. Fluorescence-activated cell sorter (FACS) based method was used for the analysis of ploidy level and the known diploid potato clone *Solanum chacoense* was used as the reference clone. The ploidy level of these cultivars was determined by comparing the value of mean peak position of G1 of reference diploid clone with mean peak position of G1 of small potato cultivars. Results indicated that all the 11 small potato cultivars collected from the Northeastern region were tetraploid with an average genome size of 837.38 Mb although they were suspected to be diploid based on the tuber characters and overall plant morphology. These cultivars were characterized by having anthocyanin pigmentation of newly emerged sprouts and eye on tuber, small and narrow leaf, long stolon length (average 7.38 cm) and high number (average 33.90/plant) of very small (average 7.41 g) tubers compared to the commercial potato varieties. However, these cultivars surpassed India's commercial tetraploid potato varieties for dry matter, starch, ascorbic acid, β-carotene and total phenol contents. Appreciably rich nutritional profile coupled with matching levels of ploidy not only enhanced the biodiversity of cultivated potato but also suggested the possibility of utilizing these cultivars in the breeding programme to enhance nutrient and antioxidant contents.

Keywords: Ploidy, genome size, flow cytometry, tuber quality, small potato, biodiversity

Introduction

Potato (*Solanum tuberosum* L.) has turned into one of the staple food of mankind because of its capacity to give high output of food per unit area (Guchi 2015). The Portuguese introduced potatoes, which they called 'Batata', to India in the early 17th century when they cultivated it along the western coast. British traders introduced potatoes to Bengal as a tuber crop and by the end of the 18th century, it was cultivated across northern hills of India (Srivastava et al. 2008). Since then, potato has been the major part of the diet in those areas, and it is traditionally cultivated in many villages. The traditional potato cultivars that have been in cultivation in the Northeastern regions of India since long differ from the modern-day potato varieties in several aspects. Among them, taste and tuber texture lead them to prefer in many local dishes. These clones are in cultivation in specific pockets of North Eastern region of India with local names and traditional knowledge of cultivation. We aim to explore and characterize those small potato cultivars' importance to local peoples as source of food and livelihood. So far, those traditional cultivars are not documented for their morphological characters, nutrient contents and ploidy level. Ploidy identification

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would be an important part in the evaluation of these unique potato germplasm resources. In the present study, we have explored and collected 11 traditional small potato cultivars from different parts of North Eastern region of India for morphological and nutritional characterization. Based on the tuber characters and overall plant morphology we suspected them to be diploid. Hence, in this study we also determined their ploidy by flow cytrometry method. Flow cytometry can quickly and accurately determine the nuclear DNA content of cells which is widely used to identify chromosome ploidy of wide range of plant species *viz*., *Rubus* species (Meng and Finn 2002), *Pisum sativum* (Dolezel and Bartos 2005), *Solanum lycopersicum* (Vera Karsburg et al. 2009), *Anacardium occidentale* (Aliyu 2012), *Erianthus arundinaceus* (Yan et al. 2016), *Urochloa* species (Tomaszewska et al. 2021), etc. Ploidy level information gives deeper in sight into the systematic of the crop (Christelová et al. 2017) which is particularly important for these "small" potato cultivars whose history of introduction and dispersal to the Northeastern region of India still remains obscure. Therefore, a study was undertaken to explore traditional small potato cultivars of North Eastern region of India to determine their ploidy level by flow cytometry method. In addition, the information on nutritional profile was also generated which may be used in breeding programme for nutritional enhancement of potato.

Materials and methods

Collection of traditional small potato cultivars

North Eastern regions of India, including northern part of West Bengal (Coochbehar and Jalpaiguri district) and Northeastern states like, Assam, Tripura, Meghalaya and Arunachal Pradesh were explored for collection of small potato cultivars which has been in traditional cultivation from long time. Tubers of 11 traditional small potato cultivars were collected and named as Jorhat Local, Coochbehar Local-1, Coochbehar Local-2, Coochbehar Local-3, Sekerkote Local, Pilak Local, Pasighat Local, Ambari Local, Upper Shillong Local, Tezu Local and Pundibari local. The passport information and geo-coordinates of the locations of collections are given in Supplementary Table 1 and Supplementary Fig 1. Around 5.0 kg tubers from each cultivar were collected from farmers' field and stored in cold storage at 2-4°C temperature. All these cultivars were first grown in the Department of Vegetable Science, Bidhan Chandra Krishi Viswavidyalaya. Tubers from the healthy plants were collected and kept in cold storage which was used for final evaluation in the next year.

Plant material and growing conditions for morphological and nutritional analysis

These 11 traditional small potato cultivars along with 3 commercial tetraploid potato varieties *viz*., Kufri Himalini, Kufri Khayti and Kufri Lalit developed at Central Potato Research Institute, Shimla, India and collected from All India Coordinated Project on Potato, Bidhan Chandra Krishi Viswavidyalaya centre were included in the evaluation trial for comparison of tuber and quality characters. These divergent cultivars were evaluated at the Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India situated at 22° 57′ N latitude and 88° 20′ E longitude at a mean sea level of 9.75 m during November to March for two consecutive years (2018-19 to 2019-2020) under open field condition with average day/night temperature of 29.32°C/16.83°C. The sandy loam inceptisol soil (pH 6.7) was thoroughly prepared and seed tubers after treating with Thiram @ 3 g·kg^{-1} were planted in randomized block design with three replications on 5 m^2 plot in each replication at 50 cm × 15 cm spacing. Cow manure @ 25 t·ha^{-1} and N: P: K was applied at 150:80:120 kg·ha^{-1}. The N was applied from urea, the P from single superphosphate and the K from muriate of potash. Half of N and all of the P and K were applied at soil preparation, and the other half of N was top dressed 40 days after planting at the time of earthing up.

Morphological characters

Five random plants per replication from each small potato cultivar and commercial tetraploid varieties were selected for recording data on different morphological characters as per DUS guidelines (PPV & FR Act 2001) *viz.,* predominant colour of light sprout, shape of light sprout, intensity of anthocyanin colouration at base of sprout, intensity of anthocyanin colouration at sprout tip, pubescence base of light sprout, length of apical sprout, foliage structure, stem solidity, stem cross section, height of main stem, predominant colour of stem, secondary colouration of stem, distribution of secondary colour on stem, leaf structure,

Fig. 1. Small potato cultivars grown under field condition including the tubers

Table 1. Flow Cytometry analysis depicting the ploidy level and genome size of the small potato cultivars

Clone name	Mean peak position of G1 (Sample)	Coefficient of variation for mean peak position of G1 (Sample)	Mean peak position of G1 ploidy reference (Solanum chacoence)	Mean peak position of G1 reference for genome size (Zea mays)	Ploidy level of small potato	2C DNA content (pg DNA)	Genome size (4x) Mb	Haploid genome size (n) Mb
Jorhat Local	43899	2.7	21574	69016	4x	3.45	3378	844.47
Cooch behar local-1	43983	2.6	21574	69016	4x	3.46	3384	846.08
Cooch behar local-2	43872	2.9	21574	69016	4x	3.45	3376	843.95
Cooch behar local-3	43921	2.6	21574	69016	4x	3.46	3380	844.89
Sekerkote local	42973	2.5	21574	69016	4x	3.38	3307	826.66
Pilak local	44528	2.9	21574	69016	4x	3.50	3426	856.57
Pasighat local	40087	2.6	21574	69016	4x	3.15	3085	771.14
Ambari local	44144	2.5	21574	69016	4x	3.47	3397	849.18
Upper Shillong local	43515	2.9	21574	69016	4x	3.42	3348	837.08
Tezu local	43834	2.0	21574	69016	4x	3.45	3373	843.22
Pundibari local	44079	2.1	21574	69016	4x	3.47	3392	847.93

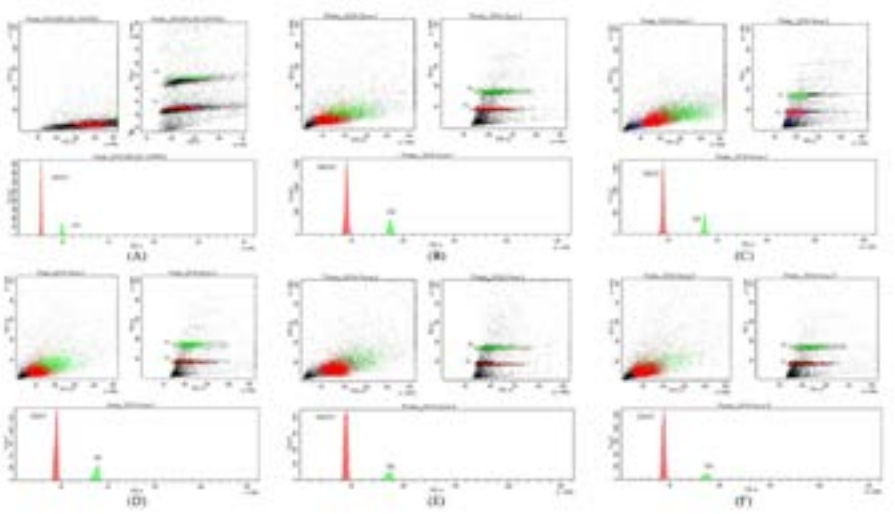

Fig. 2. Ploidy analysis using PI-fluorescence histogram of G 0 /G 1 cells of diploid control and the small potato cultivar clones

anthocyanin colouration of leaf rachis, anthocyanin colouration of leaf midrib, leaf length, leaf width, leaflet shape, waviness of leaflet margin, glossiness of upper side of leaflet, pubescence of blade at apical rosette leaflet, time of maturity of the plant, predominant skin colour of tuber, secondary skin colour of tuber, distribution of secondary skin colour of tuber, skin type of tuber, shape of tuber, depth of eye on tuber, color of eye on tuber, predominant colour of tuber flesh and secondary colour of tuber flesh. Important stolon and tuber characters recorded in these 11 small potato cultivars, along with 3 commercial tetraploid varieties at maturity from five randomly selected plants per replication, were average length of stolon (cm), average number of tuber per plant, average weight of tuber (g) and average weight of tuber per plant (g).

Tuber and tuber quality characters

These 5 randomly selected plants from each replication were utilized for recording data on average length of stolon (cm) and average number of tubers per plant. The average weight of tuber (g) was recorded from 10 randomly selected tubers per genotype per replication at harvest maturity stage. Samples of these 10 randomly selected tubers were used to estimate different quality characters *viz*., dry matter (Nissen 1955), starch (Jayaraman 1981), ascorbic acid (Besscy and King 1933), β-carotene (Glick 1957) and total phenol (Sadasivam and Manickam 1996) contents. Average data of two years for all the characters, including quality characters were subjected to statistical analysis. Variations in the characters among the genotypes were analyzed with Tukey's test using STAR software (Statistical Tool for Agricultural

Research, R-Packages, ver. 1.5 STAR 2.0.1, International Rice Research Institute, Los Banos, Philippines).

Ploidy level and genome size determination

Ploidy and genome size of these traditional small potato cultivars was estimated using flow cytometry (BD FACS Canto II, BD BioScience San Jose, CA, USA) at the Division of Crop Improvement, ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India. The external standard method was used for both analysis wherein test samples and reference standards were analyzed separately on the flow cytometer with the same instrument gain settings. Diploid *Solanum chacoense* (2*n*=2*x*=24) and *Zea mays* (2C DNA = 5.43 pg) was used as reference standard for ploidy and genome size estimation. The samples were prepared as per the methodology given by Dolezel et al. (2007) with few modifications (Kardile et al. 2020).

The modified HPI buffer containing propidium iodide (PI) was used to isolate intact nuclei. The young leaf (approx. 20 mg) was taken in an ice-cold plastic petri plate and chopped in 1-mL of modified HPI buffer (The 50 mL modified HPI buffer was prepared in distilled water by adding trisodium citrate dihydrate (0.05 gm), triton X-100 (150 µL), RNase (2.5 mL), PVP (0.5 gm). The solution was sterilized using filter sterilization, and β-mercaptoethanol (15 µL) and PI (1.25 mL) were added to the filtrate) using surgical blades under dark conditions. The samples were then filtered through the 40 µm filters. The homogenate was incubated for 15 minutes on ice under the dark condition with occasional shaking to allow the PI to bind to the DNA of the isolated nuclei. Then, the samples were analyzed for DNA ploidy and genome size estimation using diploid, *Solanum chacoense*, as reference DNA standards, respectively. The methodology for preparing intact nuclei suspension for reference standard remains the same as that of test samples.

The stained nuclei of the test sample and reference standard were run through the flow cytometer with an excitation wavelength of 480 nm band pass filter. Ten thousand PI cell signals were detected per sample, and PI-fluorescence histograms were generated to estimate the ploidy and genome size. The data were analyzed using the BD FACS Diva software and ploidy and genome size were calculated using the following formulae (Dolezel et al. 2007).

$$\text{Sample ploidy (integer)} = \text{Reference ploidy} \times \frac{\text{Mean position of G1 sample peak}}{\text{Mean position of G1 reference peak}}$$

$$\text{Sample 2C value (DNA pg or Mbp)} = \text{Reference 2C value} \times \frac{\text{Mean position of G1 sample peak}}{\text{Mean position of G1 reference peak}}$$

Estimated genomic content in picogram (pg) has been converted to million base pair (Mb) for simplicity.

Results and discussion

Ploidy level and genome size estimation

The phenomenon of polyploidy in plants has long interested plant breeders (Spooner et al. 2010). Flow cytometry has become the standard method of ploidy estimation (Dolezel and Bartos 2005) which measures the light scattered from particles. DNA-specific fluorochromes are used to label nuclei, which release longer-wave length fluorescence after being excited by light (Bohanec 2003). The sorted cell populations were plotted on the histogram with PE (R-phycoerythrin) area versus cell count in the present investigation. For known diploid reference clone, *Solanum chacoense*, DNA peak of G0/G1 cells was plotted at 21574 (Mean of PE area) with 1,455 cell count. The CV of the G1 peak was very low of 2.3% which is desirable (Tomaszewska et al. 2021). Ploidy estimation results (Table 1 and Fig 2) showed that all the eleven tested small potato cultivars of the Northeastern region of India were tetraploid with an average genome size of 837.38 Mb, which is consistent with the reported genome size of potato (Xu et al, 2011). Interestingly, the clone Pasighat local has a comparatively smaller (771.14 Mb) genome size than the rest of the clones. However, we suspected them to be diploid based on long stolon, small tuber characters and overall plant morphology. Our results suggested that the fluorescence-activated cell sorter (FACS) based method provided an adequate and applicable estimate of ploidy level and genome size of the traditional small potato cultivars. G1 peak CV% was low, ranging between 2.0 to 2.9% which depicted sharper and thinner peak of both sample (small potato cultivars) and reference standard (*Solanum chacoense*).

Morphological characterization

Morphological characterization of the cultivars not only helps in the identification of the clones with unique characteristics but also in the planning for inheritance studies (Darkwa et al. 2020). However, no information is available so far on the morphological characterization of small potato cultivars traditionally grown in the Northeastern regions of India. The small potato genotypes under study varied widely among each other (Fig. 1). Thirty-two DUS characters have been considered to characterize these traditional small potato cultivars (Supplementary Table 2). Newly emerged sprouts of all the cultivars showed anthocyanin pigmentation, producing purple, pink, or red-purple coloration. The presence or absence of red-purple pigmentation is controlled by a number of single genes (Brown 2005). Light sprouts of most of the cultivars were either cylindrical or spherical and only 2 had conical-shaped light sprouts. The intensity of anthocyanin coloration at the base of the sprout was dark in most of the cultivars, followed by light in 3 cultivars and medium in 2 cultivars. The intensity of anthocyanin coloration at the tip of the sprout was medium in most of the cultivars, light in 2 cultivars and dark in 1 cultivar. Variation in anthocyanin pigmentation among the cultivars might be due to varied expression of highly homologous gene *StMYBA1* / *StAN2* (Strygina et al. 2019). Most of the cultivars had weak pubescence in the light

Table 2. Mean of different small potato genotypes and commercial ware potato varieties

Genotype	Length of stolon (cm)	Number of tuber per plant	Average weight of tuber (g)	Dry matter content (%)	Starch content (%)	β-carotene content (µg/100 g fresh)	Ascorbic acid content (mg/100 g fresh)	Total phenol content (mg GAE/ 100 g fresh)
Small potato cultivars								
Jorhat Local	7.25^{c}	22.19^{ef}	2.86^{h}	23.69^{cd}	24.07^{a}	100.38^{b}	37.35^{ab}	16.93^{dc}
Coochbehar Local -1	8.49^{cb}	28.02^{ed}	5.03^{f}	24.30^{cb}	22.12^{bc}	69.57^{c}	33.84^{bc}	17.74^{b}
Coochbehar Local -2	8.48^{cb}	21.55^{f}	10.28^{e}	23.19^{d}	22.37^{bc}	71.14^{c}	36.38^{ba}	18.39^{a}
Coochbehar Local -3	14.34^{a}	92.08^{a}	1.63^{h}	23.64^{cd}	22.16^{c}	49.89^{e}	26.99^{gf}	18.18^{a}
Sekerkote Local	3.49^{d}	24.69^{edf}	2.83^{h}	27.22^{a}	15.99^{gf}	100.53^{b}	32.56^{dc}	16.36^{e}
Pilak Local	3.88^{d}	21.11^{f}	11.93^{e}	26.74^{a}	16.83^{ef}	60.92^{d}	31.54^{dce}	16.93^{dc}
Pasighat Local	9.41^{cb}	54.89^{b}	4.80^{f}	24.84^{b}	17.57^{ed}	107.16^{a}	24.83^{g}	17.10^{c}
Ambari Local	3.72^{d}	20.08^{f}	17.27^{d}	24.21^{cb}	23.33^{ba}	103.21^{ba}	29.58^{dfe}	16.64^{de}
Upper Shillong Local	3.65^{d}	19.84^{g}	17.97^{d}	20.60^{g}	15.25^{g}	37.92^{f}	27.62^{gf}	15.99^{f}
Tezu Local	10.65^{b}	38.87^{c}	4.04^{gf}	23.00^{ed}	16.78^{ef}	31.50^{gh}	26.59^{gf}	16.38^{e}
Pundibari Local	7.82^{cb}	29.68^{d}	2.81^{gh}	22.41^{e}	22.60^{bac}	69.62^{c}	28.60^{fe}	18.13^{a}
Mean	7.38	33.90	7.41	23.98	19.91	72.89	30.53	17.16
Commercial tetraploid potato varieties								
Kufri Himalini	2.31^{d}	10.55^{g}	50.01^{b}	20.62^{gf}	18.91^{d}	36.82^{gf}	20.39^{h}	15.14^{g}
Kufri Khayti	2.46^{d}	7.88^{g}	57.33^{a}	19.57^{h}	17.67^{ed}	33.09^{gfh}	21.06^{h}	14.31^{h}
Kufri Lalit	3.63^{d}	9.65^{g}	37.43^{c}	21.34^{f}	18.66^{d}	30.16^{h}	20.39^{h}	14.92^{g}
Mean	2.80	9.36	48.25	20.51	18.41	33.35	20.61	14.79
C.V.^{a} (%)	14.63	10.32	3.96	2.21	3.02	2.58	6.04	4.39

C.V. = Coefficient of variation.

Among different characters, values followed by different lower case letters are significantly different at $p \leq 0.05$ by Tukey's HSD (honest significant difference) test (otherwise statistically at par)

sprout and only 2 cultivars showed strong pubescence at the base of the sprout. Apical sprout was small in most of the cultivars while it was medium in 2 cultivars. Variation of apical sprouting among the genotypes might be due the difference in expression of *StPP2Ac2b*, a catalytic subunit of protein phosphatase 2A (García et al. 2022). The foliage structure was semi-compact in most of the cultivars, open in 3 cultivars and compact in only 1 cultivar. Most of the cultivars had solid stems while only 3 cultivars had hollow stem. Cross section of the stem was round for most of the cultivars and only 3 cultivars had angular cross section of the stem. In most of the cultivars, height of the above-ground main stem was short while it was medium only in 1 cultivar. Anthocyanin pigmentation in the newly emerged sprouts disappeared with the advancement of age and stem of most of the cultivars was green while only 5 cultivars retained purple coloration on the stem. The cultivars which retained the anthocyanin may be used to develop promising source of high value phytochemicals for food industry (Jeddou et al. 2021). There was a close correspondence in anthocyanin pigmentation of the rachis and midrib of leaf among the genotypes which retained anthocyanin in the stem. The cultivars in which anthocyanin pigmentation disappeared in the stem did not show coloration in the rachis and midrib of leaf. The predominant leaf structure was open type while, it was intermediate in 4 cultivars. Most of the cultivars had small leaf length and width while two cultivars had broad leaves. In most of the cultivars, upper side of the leaflets was glossy. The glossiness is due to the presence of cuticular waxes whose main constituents are very long-chain n-alkanes, 2- and 3-methylalkanes, primary alcohols, fatty acids and wax esters (Szafranek et al. 2008). Variation in cuticular wax compositions among the genotypes stimulates or dissuades insect oviposition, feeding or movement (Li and Ishikawa 2006). Pubescence of leaf blade at apical rosette of the leaflet was absent in most of the cultivars and present in 4 cultivars. Earlier studies suggest that presence of pubescence in leaf has a positive correlation with pest and disease resistance (Gurr and McGrath 2002; Alizadeh-Moghaddam et al. 2022). Most of the cultivars fell under late maturity group while 2 cultivars were in medium maturity group. Anthocyanin pigments have been deposited in the tuber skin of most of

the cultivars producing different intensities of violet or red coloration while it was absent in rest of the 2 cultivars which produced whitish-cream or yellow skin color in the tuber. Anthocyanin pigmentation in tuber skin is controlled by a dominant allele in *D* locus located in chromosome 2 (Brown 2005). The whitish-cream or yellow-skin colored tuber lacked the functional allele of *D* (Jung et al. 2009). Anthocyanin pigmentation was predominant in the tuber skin but not in tuber flesh in most of the cultivars which might be due to *I* locus epistatically controlling pigmentation in tuber skin and tuber flesh even when *D* is present (Brown 2005). In most of the cultivars, tuber-flesh was cream in color. Tuber skin type was smooth in 8 cultivars while, tuber skin type was rough in 3 cultivars. Most of the cultivars produced round shaped tuber, 2 each cultivar produced long-oblong and flattened tuber. Earlier reports suggest that alleles conferring round tuber shape are dominant over alleles producing long-oblong and flattened tuber (Van Eck et al. 1994; Prashar et al. 2014) Five cultivars had shallow depth of tuber eye while, the rest 6 cultivars produced tuber with either medium deep eye (4 cultivar) or deep eye (2 cultivar). Tuber form and eye depth are two of the many potato tuber qualities that breeders should focus on for fresh market applications and for the potato processing business. QTL for tuber eye depth is closely linked with QTL for tuber shape and is under monogenic control (Li et al. 2005). Close correspondence was apparent between pigmentation pattern in the skin and eye of the tuber. Color of the eye of most of the cultivars was purple. One cultivar with whitish-cream tuber skin color also had similar eye colour.

Tuber characters and nutritional profile

The mean of 3 tuber and 5 tuber quality characters varied significantly among the traditional small potato cultivars and commercial potato varieties (Table 2). Stolon length varied significantly among the cultivars and it was much higher in the small potato cultivars (average 7.38 cm) than the commercial potato varieties (average 2.80 cm). Significant variation in stolon length might be due to calcium-dependent protein kinases which are found in abundance in early elongating stolons (Yousaf et al. 2021). The small potato cultivars produced about 3.5 times more tuber number per plant (average 33.90 tubers /plant) compared to commercial potato varieties (average 9.35 tubers/plant). The average weight of tuber varied significantly among the genotypes and was 6.5 times less in small potato cultivars (average 7.41g) than in commercial potato varieties (average 48.25 g). In spite of significant variation in tuber weight, all the traditional small potato cultivars were considered as members of the same group because of their specific taste and similarity in culinary purpose. The average weight of tuber appeared to be inversely related to the length of stolon and the number of tubers per plant, which corroborated with the earlier findings of Solomon et al. (2019). The small potato cultivars appeared to be nutritionally rich which surpassed the commercial potato varieties for all the nutrient and antioxidant contents *viz*., dry matter (16.91% high), starch (8.14% high), ascorbic acid (48.13% high), β-carotene (118.56% high) and total phenol contents (19.16% high).

The main difference between traditional small potato cultivars of Northeastern India and improved tetraploid cultivars are small and narrow leaf *vs* medium to large leaf, medium to strong waviness of leaf let margin *vs* weak waviness, long stolon *vs* short stolon, a large number of small tubers *vs* few numbers of medium to big tubers, high antioxidant and nutrient content *vs* comparatively low antioxidant and nutrient content. These characters can be utilized in the breeding programme. The appreciably rich nutritional profile of small potato cultivars compared to the commercial tetraploid potato varieties, particularly with respect to ascorbic acid and β-carotene contents, coupled with matching levels of ploidy not only increased the biodiversity of cultivated potato but also suggested the possibility of their involvement in a conventional breeding programme for enhancement of nutrient and antioxidant contents.

Supplementary material

Supplementary Tables S1 to S4 and Supplementary Fig. 1 are provided on www.isgpb.org

Author's contributions

Conceptualization of research (PH); Designing of the experiments (BP, PH, AC); Contribution of experimental materials (AKC, HBK); Execution of field/lab experiments and data collection (BP, SH); Analysis of data and interpretation (SKC, AM); Preparation of the manuscript (SH, PH)

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Supplementary Table 1. Passport information of small potato

S. No.	Collected Germplasm	Crop Name	Common Name	Source	Place of origin District / State	Biological status	Location	Geo-Coordinates
01.	Jorhat Local	Potato	Badam aloo	Farmer	Jorhat / Assam	Landraces	Jorhat	26.7509° N 94.2037° E
02.	Coochbehar Local 1	Potato	Choto Allu	Farmer	Coochbehar / West Bengal	Landraces	Sajerpar	26.4222° N 89.3529° E
03.	Coochbehar Local 2	Potato	Lal Allu	Farmer	Coochbehar / West Bengal	Landraces	Gopalpur	26.4242° N 89.4505° E
04.	Coochbehar Local 3	Potato	Choto Allu	Farmer	Coochbehar / West Bengal	Landraces	Khagrabari	26.3504° N 89.4435° E
05.	Sekerkote local	Potato	Choto Allu	Farmer	West Tripura / Tripura	Landraces	Sekerkote	23.7387° N 91.2710° E
06.	Pilak Local	Potato	Lal Aloo	Farmer	SouthTripura / Tripura	Landraces	Pilak	23.2065° N 91.6039° E
07.	Pasighat local	Potato	Allo	Farmer	East Siang / Arunachal Pradesh	Landraces	Pasighat	28.0618° N 95.3259° E
08.	Ambari local	Potato	Allo	Farmer	Jalpaiguri / West Bengal	Landraces	Ambari	26.6090° N 88.4864° E
09.	Upper shillong local	Potato	Phan	Farmer	East Khasi Hills / Meghalaya	Landraces	Upper shillong	25.5840° N 91.9003° E
10.	Tazu local	Potato	Allo	Farmer	Lohit / Arunachal Pradesh	Landraces	Tazu	27.9277° N 96.1533° E
11.	Pundibari local	Potato	Allu	Farmer	Coochbehar / West Bengal	Landraces	Pundibari	26.5243° N 89.1075° E

Supplementary Table 2. Morphological characterization of genotypes based on 32 DUS characters

a. Sprout character

Cultivar	Predominant colour of light sprout	Shape of light sprout	Intensity of anthocyanin colouration at base of sprout	Intensity of anthocyanin colouration at sprout tip	Pubescence base of light sprout	Length of apical sprout
Small potato cultivars						
Jorhat Local	Purple	Cylindrical	Dark	Medium	Weak	Small
Coochbehar local-1	Purple	Spherical	Dark	Medium	Weak	Small
Coochbehar local-2	Purple	Conical	Dark	Medium	Weak	Small
Coochbehar local-3	Purple	Cylindrical	Dark	Medium	Weak	Small
Sekerkote local	Pink	Cylindrical	Light	Medium	Strong	Small
Pilak local	Pink	Spherical	Medium	Medium	Weak	Small
Pasighat local	Pink	Spherical	Medium	Medium	Weak	Small
Ambari local	Purple	Spherical	Dark	Light	Weak	Small
Upper Shillong local	Red Purple	Cylindrical	Light	Light	Strong	Medium
Tezu local	Red Purple	Spherical	Light	Medium	Weak	Small
Pundibari local	Purple	Cylindrical	Dark	Medium	Weak	Small
Commercial tetraploid potato varieties						
Kufri Himalini	Green	Cylindrical	Green	Green	Weak	Medium
Kufri Khayti	Green	Conical	Green	Green	Weak	Medium
Kufri Lalit	Pink	Spherical	Dark	Dark	Absent	Small

Supplementary Table 3. Morphological characterization of genotypes based on 32 DUS characters.
b. Foliage and stem characters

Cultivar	Foliage structure	Stem solidity	Stem cross section	Height of main stem	Predominant colour of stem	Secondary colouration of stem
Small potato cultivars						
Jorhat Local	Semi-Compact	Solid	Round	Short	Purple	Green
Cooch behar local-1	Semi-Compact	Solid	Round	Short	Purple	Green
Cooch behar local-2	Semi-Compact	Solid	Angular	Short	Purple	Green
Cooch behar local-3	Semi-Compact	Solid	Angular	Short	Purple	Green
Sekerkote local	Open	Solid	Angular	Short	Green	Purple
Pilak local	Semi-Compact	Solid	Round	Short	Green	Purple
Pasighat local	Semi-Compact	Solid	Round	Short	Green	Absent
Ambari local	Open	Hollow	Round	Medium	Green	Purple
Upper Shillong local	Open	Solid	Round	Short	Green	Absent
Tezu local	Semi-Compact	Solid	Round	Short	Green	Purple
Pundibari local	Semi-Compact	Solid	Round	Short	Purple	Green
Commercial tetraploid potato varieties						
Kufri Himalini	Compact	Hollow	Round	Short	Green	Absent
Kufri Khayti	Semi-Compact	Hollow	Round	Short	Green	Absent
Kufri Lalit	Semi-Compact	Solid	Round	Short	Green	Purple

Supplementary Table 4. Morphological characterization of genotypes based on 32 DUS characters.
c. Stem and leaf characters

Cultivar	Distribution of secondary colour on stem	Leaf structure	Anthocyanin colouration of leaf rachis	Anthocyanin colouration of leaf midrib
Small potato cultivars				
Jorhat Local	Throughout lightly scattered	Open	Present	Present only at the base
Cooch behar local-1	Throughout lightly scattered	Open	Present	Present only at the base
Cooch behar local-2	Throughout lightly scattered	Open	Present	Present only at the base
Cooch behar local-3	Throughout highly scattered	Open	Present	Present only at the base
Sekerkote local	Throughout lightly scattered	Open	Absent	Absent
Pilak local	Throughout lightly scattered	Intermediate	Absent	Absent
Pasighat local	Absent	Open	Absent	Absent
Ambari local	Only at lower node	Intermediate	Absent	Absent
Upper Shillong local	Absent	Intermediate	Absent	Absent
Tezu local	Throughout lightly scattered	Open	Absent	Absent
Pundibari local	Throughout lightly scattered	Open	Present	Present only at the base
Commercial tetraploid potato varieties				
Kufri Himalini	Absent	Open	Absent	Absent
Kufri Khayti	Absent	Intermediate	Absent	Absent
Kufri Lalit	Only at base	Open	Absent	Absent

Supplementary Table 2: Morphological characterization of genotypes based on 32 DUS characters.
d. Morphological characterization of genotypes: Leaf and leaflet characters

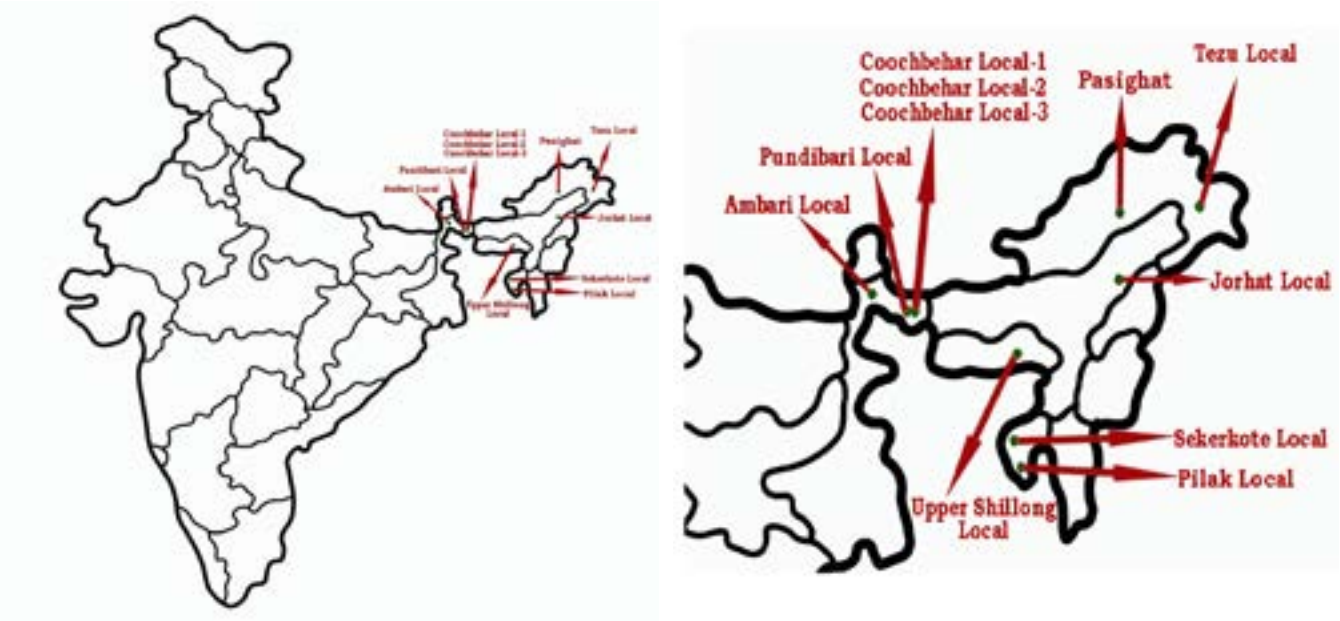
Cultivar	*Leaf length*	*Leaf width*	*leaf let shape*	*Waviness of leaf let margin*	*Glossiness of upper side of leaflet*	*Pubescence of blade at apical rosette leaflet*
Small potato cultivars						
Jorhat Local	Small	Narrow	Narrow lanceolate	Strong	Medium	Present
Cooch behar local-1	Small	Narrow	Ovate lanceolate	Medium	Medium	Present
Cooch behar local-2	Small	Narrow	Lanceolate	Medium	Medium	Present
Cooch behar local-3	Small	Narrow	Narrow lanceolate	Strong	Medium	Present
Sekerkote local	Small	Narrow	Lanceolate	Strong	Weak	Absent
Pilak local	Medium	Narrow	Ovate	Medium	Weak	Absent
Pasighat local	Medium	Medium	Ovate lanceolate	Medium	Strong	Absent
Ambari local	Large	Broad	Ovate	Medium	Strong	Absent
Upper Shillong local	Small	Narrow	Ovate lanceolate	Strong	Strong	Absent
Tezu local	Small	Narrow	Ovate lanceolate	Medium	Medium	Absent
Pundibari local	Small	Narrow	Narrow lanceolate	Medium	Medium	Absent
Commercial tetraploid potato varieties						
Kufri Himalini	Large	Medium	Ovate lanceolate	Weak	Strong	Absent
Kufri Khayti	Medium	Medium	Ovate lanceolate	Weak	Strong	Absent
Kufri Lalit	Medium	Broad	Ovate	Weak	Strong	Absent

Supplementary Table 2: Morphological characterization of genotypes based on 32 DUS characters.
e. Tuber characters

Supplementary Table 2: Morphological characterization of genotypes based on 32 DUS characters.	*Time of maturity of the plant*	*Predominant skin colour of tuber*	*Secondary skin colour of tuber*	*Distribution of secondary skin colour of tuber*	*Skin type of tuber*	*Shape of tuber*
Small potato cultivars						
Jorhat Local	Late	Purple	Absent	Absent	Smooth	Oblong
Cooch behar local-1	Late	Purple	Absent	Absent	Smooth	Round
Cooch behar local-2	Late	Purple	Absent	Absent	Smooth	long-oblong
Cooch behar local-3	Late	Pink	Absent	Absent	Smooth	Round
Sekerkote local	Medium	Pink	Whitish cream	Splashed	Smooth	Round
Pilak local	Late	Red	Whitish cream	Splashed	Rough	Round
Pasighat local	Late	Whitish cream	Purple	Confined to eyes	Smooth	Round
Ambari local	Medium	Red	Whitish cream	Splashed	Rough	Ovoid
Upper Shillong local	Early	Whitish cream	Absent	Absent	Smooth	Round
Tezu local	Late	Whitish cream	Red	Confined to eyes	Rough	Round
Pundibari local	Late	Purplish Red	Absent	Absent	Smooth	long-oblong
Commercial tetraploid potato varieties						
Kufri Himalini	Medium	Yellow	Absent	Absent	Smooth	Flattened
Kufri Khayti	Early	Whitish cream	Absent	Absent	Smooth	Flattened
Kufri Lalit	Medium	Red	Pink	Confined to eyes	Smooth	Round

Supplementary Table 2. Morphological characterization of genotypes based on 32 DUS characters.
f. Tuber eye and flesh characters

Cultivar	Depth of eye on tuber	Colour of eye on tuber	Predominant colour of tuber flesh	Secondary colour of tuber flesh
Small potato cultivars				
Jorhat Local	Shallow	Purple	Cream	Reddish purple
Cooch behar local-1	Medium deep	Purple	Cream	Reddish purple
Cooch behar local-2	Shallow	Purple	Cream	Reddish purple
Cooch behar local-3	Medium deep	Purple	Cream	Reddish purple
Sekerkote local	Medium deep	Purple	Cream	Absent
Pilak local	Deep	Purple	Cream	Absent
Pasighat local	Shallow	Purple	Yellow	Absent
Ambari local	Deep	Purple	Cream	Absent
Upper Shillong local	Shallow	Whitish cream	White	Absent
Tezu local	Medium deep	Purple	Cream	Absent
Pundibari local	Shallow	Purple	Cream	Reddish purple
Commercial tetraploid potato varieties				
Kufri Himalini	Shallow	Yellow	Cream	Absent
Kufri Khayti	Shallow	Whitish cream	Cream	Absent
Kufri Lalit	Shallow	Purple	Cream	Absent



Supplementary Fig. 1. Indian Map shown the location of collected germplasm