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DEVELOPMENT OF NITROGEN USE EFFICIENT POTATO HYBRIDS: EVALUATION OF PROGENIES FOR AGRONOMIC, PHYSIO-BIOCHEMICAL AND NITROGEN USE EFFICIENCY TRAITS

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ABSTRACT: Global agriculture is causing environmental pollution due to excess application of nitrogen (N) fertilizers in high N requiring crops like potato. Hence, aim of this study was to develop potato hybrids with enhanced nitrogen use efficiency (NUE) and yield traits under limited N availability. We generated here a bi-parental population of 116 progenies by crossing two contrasting varieties *viz.*, Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient). After six years (2015-21) of breeding, clonal selection and field trials, we developed advance hybrids based on 20 traits of agronomic, physio-biochemical and NUE parameters. Significant variations were observed in the progenies for most traits such as plant height, total leaf area, shoot dry weight and tuber traits (number, yield and dry matter). Further, plant total N content, NUE variables (NUE, NUpE, NUtE, HI and NHI) and physio-biochemical properties also differed significantly. The principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) distinguished variations. We observed significant and positive correlations with tuber traits and NUE variables. Finally, three advance hybrids (NUE/15-8, NUE/15-23, and NUE/15-67) with high yield and NUE combined with other desirable tuber traits are demonstrated here. Our study also implies that tuber yield and NUE are the key criteria for selection of N use efficient genotypes under limited N input.

KEYWORDS: Breeding, nitrogen use efficiency, potato, hybrid, yield

INTRODUCTION

In the 21st century, food availability is a serious challenge for more than 9 billion population by 2050 in the world (United Nations, 2015). The situation would be more severe in developing countries where population growth is rapid, cultivable land resources are limited and soil fertility is degrading due to excess application of N fertilizers in crops (St. Clair and Lynch 2010). Potato is the third most important food crop and it has tremendous potential to meet food and nutritional security in the world. Nitrogen is an important macronutrient for plant growth, yield and tuber quality of potato. Potato prefers nitrate form of nitrogen than ammonium, and it is prone to nitrate leaching (Goffart *et al.*, 2008). The situation is more aggravated due to irrigated cultivation and shallow rooted nature of potato crop. N loss in the form of volatalisation of ammonia or nitrous oxide (N_2O) gases causing greenhouse gas emission is the main problem of N fertilization (Vos, 2009). High N application also causes increase in production cost and deteriorates health of soil, water resources and air quality. Presently, more than 100 million tonnes of N fertilizers are applied to crops in fields, of which about 3 million tonnes in root and tuber crops including potato (FAO, 2015). Since, less than half of N is utilized by crops and the remaining N is lost in environment, there is a need to improve plant nitrogen use efficiency (NUE) (Garnett *et al.*,

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2015). Therefore, global agriculture urgently requires technology for limited resources, in which, development of nitrogen use efficient potato cultivars is one of the environment friendly options for efficient use of nitrogen.

In potato growing countries, different N doses are applied to crops to obtain high yields (30-50 t ha^{-1}) such as 90-240 kg N ha^{-1} (India), 120-160 kg N ha^{-1} (Germany) and 40-270 kg N ha^{-1} (UK) (Koch *et al.* 2020). Under Indian sandy-loam soil conditions, potato crop removes 120-140 kg N ha^{-1} from the soils to produce tuber yield of 25-30 tonnes ha^{-1} (Trehan and Singh, 2013). To address NUE, soil and agronomic manipulation strategies have been applied world over in potato through site-specific nutrient management to increase N fertilizer use efficiency (Zebarth and Rosen, 2007; Vos 2009). Although, genetic variability has been analysed under field experiments for yield and NUE traits in potato such as varieties (Zebarth *et al.*, 2004; Trehan, 2009; Trehan and Singh, 2013; Ospina *et al.*, 2014), advance clones (Sharifi *et al.*, 2007), and germplasm (Sattelmacher *et al.*, 1990; Errebhi *et al.*, 1998, 1999; Zebarth *et al.*, 2008); yet, development of potato varieties with enhanced NUE is meagre, except a few (Ospina *et al.*, 2014; Tiemens-Hulscher *et al.*, 2014). This could be due to that NUE is a complex trait and tetraploid potato combined with NUE is more affected by soil conditions, genotypes, environment and its measurement parameters. Exploitation of genetic variation has been suggested for improving NUE in cereal crops (Hawkesford and Griffiths, 2019). Moreover, with the increasing knowledge in rice, wheat and maize, NUE research has gained more interest on agronomy, breeding and biotechnology aspects (Garnett *et al.*, 2015; Ranjan and Yadav, 2019; Silva *et al.*, 2020). A study indicates progress in cereals, vegetables and oil seed crops including potato on implication of cultural practices, cultivars and breeding for NUE (van Bueren and Struik, 2017). Recently, we have highlighted application in integrated genomics, physiology and breeding approaches for improving NUE in potato (Tiwari *et al.*, 2018).

The aim of this study was to develop N-use efficient potato hybrids through conventional breeding and clonal selection methods. In the present study a total of 116 progenies of a cross involving Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient) were developed, which further led to development of N use efficient potato hybrids based on 20 agronomic, physio-biochemical and NUE traits for low N input agriculture.

MATERIALS AND METHODS

Plant materials

Two contrasting potato varieties viz., Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient) were selected based on previous field studies (Trehan, 2009; Trehan and Singh, 2013). A bi-parental population of 116 segregating progenies (Kufri Jyoti × Kufri Gaurav) were generated in 2015 at Indian Council of Agricultural Research (ICAR)-Central Potato Research Institute (CPRI), Kufri, Shimla, Himachal Pradesh, India (31.09°N and 77.26°E; and 2720 m amsl) under natural flowering in hill conditions. Then, seedling raising from true potato seed (TPS) and clonal selections were performed under field conditions at ICAR-CPRI, Regional Station, Modipuram, Uttar Pradesh, India (29.06°N and 77.71°E; and 237 m amsl) under sub-tropical plain regions, which cover more than 80% of potato growing area in the country. The soil texture was sandy loam with neutral pH (7.01) and had low organic carbon (0.19%) with available N (160.2 kg ha^{-1}), P (26.7 kg ha^{-1}) and K (126.3 kg ha^{-1}). The progenies (116) were advanced after clonal selection during 2015-2020. Initially, due to limited tuber numbers, clonal selection trials of the progenies were conducted in two-rows trials (15 × 2 = 30 tubers of each genotype per replication, spacing: 60 × 20 cm) with randomized block design in two replications during 2017-18 and 2018-19 under limited nitrogen supply (50 kg N ha^{-1}) till 90 days after planting. The recommended dose of N in this region is 180 kg N ha^{-1}. Total 118 genotypes (116 progenies and 2 parents, as control) were evaluated for agronomic, physio-biochemical, NUE parameters and yield traits, and advance hybrids with enhanced NUE were selected for further multi-location field trials. All traits were measured from at least three plants per replication. Three selected hybrids were evaluated under a large field trial (three replications) with different N doses (0, 50 and 180 kg N ha^{-1}) of eight-rows (15 × 8 = 120 tubers of each genotype per replication) at same spacing (60 × 20 cm^2) for two years (2019-20 and 2020-21) till 90 DAP.

Measurement of phenotypic and yield traits

Plant height (cm) and total leaf area (cm^2) were measured on per plant basis from 118 genotypes at 65 DAP at good vegetative growth stage. Total leaf area was measured by using the LI-3100C Area Meter (LICOR Biosciences, Lincoln, Nebraska, USA). Shoot dry weight (g), tuber number and tuber yield (g) were also estimated on per plant basis at harvest stage (90 DAP). Dry weight of shoot samples was measured after hot air oven drying at 70°C for 4-5 days (Binder, Tuttlingen, Germany).

Estimation of total chlorophyll, total N and NUE parameters

Total chlorophyll content (SPAD value) in 118 genotypes was measured in the 4th leaf from top using the SPAD-502Plus (KONICA MINOLTA, INC. USA). Plant total N was estimated from dried samples (shoots and tubers) of 118 genotypes (two replications) following Kjeldahl method described by Singh *et al.* (2005). Briefly, plant samples were digested in sulphuric acid at a temperature between 360-410°C. The rate of digestion was accelerated by using copper sulphate as a catalyst, and anhydrous sodium sulphate or potassium sulphate to raise the boiling temperature of H_2SO_4. On completion of digestion, the samples were cooled and diluted as concentrated alkali was to be added to H_2SO_4 digest for distillation. The distilled ammonia is quantitatively absorbed in boric acid and titrated against the standard acid (H_2SO_4). NUE variables (AgNUE, NUE, NUpE, NUtE, HI and NHI) were calculated using the original formula given by Zebarth *et al.* (2004, 2008), as recently applied in potato (Tiwari *et al.*, 2020). Minimum of three plants per replication were used for estimation.

$$\text{Total N (\%)} = \frac{0.0014 \times (\text{Titre value - Blank value})}{\text{Sample weight}}$$

Where, 0.0014 = Factor (1 ml of 0.1 N H_2SO_4 = 0.0014 g N)

$$\text{Agronomic nitrogen use efficiency (AgNUE)} = \frac{\text{Fresh tuber yield (g)}}{\text{Crop N supply (kg)}}$$

$$\text{Nitrogen use efficiency (NUE)} = \frac{\text{Plant dry matter accumulation (g)}}{\text{Crop N supply (kg)}} = \text{NUpE} \times \text{NUtE}$$

$$\text{Nitrogen uptake efficiency (NUpE)} = \frac{\text{Plant N accumulation (g)}}{\text{Crop N supply (kg)}}$$

$$\text{Nitrogen utilisation efficiency (NUtE)} = \frac{\text{Plant dry matter accumulation (g)}}{\text{Plant N accumulation (g)}}$$

$$\text{Harvest index (HI)} = \frac{\text{Tuber dry matter accumulation (g)}}{\text{Plant dry matter accumulation (g)}}$$

$$\text{N harvest index (NHI)} = \frac{\text{Tuber N accumulation (g)}}{\text{Plant N accumulation (g)}}$$

Estimation of biochemical parameters

Biochemical parameters were estimated in 118 genotypes for traits such as amino acids, total soluble proteins, reducing sugars, sucrose, total soluble sugars, and ascorbic acids. Total free amino acids were estimated as per the method described by Thimmaiah (2009). Fresh tuber sample (10 g) was processed with 10 ml of 80 % methanol. Sample was centrifuged and residue was re-extracted with 80 % methanol followed by centrifugation. Supernatants were pooled and volume of supernatant was raised to 20 ml. Aliquot (100 µl) was raised to 1 ml with methanol. Ninhydrin solution (5 ml) was added to the sample, vortexed, heated in boiling water bath for 10 min and cooled to room temperature. Optical density was recorded at 570 nm.

For extraction of reducing sugars and sucrose, potato flesh (10 g) was made protein free by using lead acetate and potassium oxalate. The samples were filtered and volume was raised to 20 ml with distilled water. Reducing sugars were determined by the method developed by Somogyi (1952). For estimation of reducing sugars, 100 µl aliquot was raised to 1 ml with distilled water. After adding 1 ml Nelson alkaline reagent the samples were boiled for 20 min and cooled in chilled water. Nelson's arsenomolybedate reagent (1 ml) was added and samples were vortexed. Distilled water (7 ml) was added and optical density was measured at 620 nm. The method described by Van Handel (1968) was followed for the estimation of sucrose content. 100 µl of 30% potassium hydroxide was added to the samples (100 µl aliquot + 900 µl distilled water). The samples were boiled for 10 min and cooled in chilled water. After bringing the samples to room temperature, 3 ml of 0.15% anthrone solution prepared in 76% sulphuric acid was added. The samples were incubated at 40°C for 15 min and optical density was measured at 620 nm.

Total soluble sugars were estimated in the samples as per the method described by Thimmaiah (2009). Sample (1 g) was hydrolyzed with 5 ml of 2.5N hydrochloric acid for 3 h in boiling water bath. After incubation, samples were neutralized with sodium carbonate. Volume of the samples was raised to 100 ml with distilled water. Aliquot (100 *µl) was raised to 1ml with distilled water.* 4 ml of 0.2% Anthrone reagent prepared in chilled sulphuric acid was added and incubated for 8 min in boiling water bath. Samples were cooled rapidly in chilled water and optical density was recorded at 630 nm.

Total soluble proteins were estimated with the method of Lowry *et al.* (1951). Dry sample (1 g) was extracted with 5 ml of 0.1 N sodium hydroxide, vortexed and centrifuged at 10000 rpm, for 10 min. Volume of supernatant was raised to 5 ml with 0.1 N sodium hydroxide. One ml of 15% trichloro-acetic acid was added and samples were incubated at 4°C for 24h. Samples were centrifuged at 5000 rpm for 20 min. Residue/precipitates were dissolved in 5 mL of 0.1 N sodium hydroxide. Aliquot (0.5 ml) was mixed with 5 ml of solution prepared by mixing 50 ml of 2% sodium carbonate in 0.1 N sodium hydroxide with 1 ml of .5% $CuSO_4{\cdot}5H_2O$ in 1% sodium potassium tartarate. Samples were vortexed and incubated for 5 min. 0.5 ml of Folin-Ciocalteu phenol reagent (prepared by mixing this reagent and distilled water in equal quantity at time of use) was added and incubated for 60 min at room temperature. Optical density was measured at 570 nm.

Extraction and estimation of ascorbic acid was carried out as per method described by Stan *et al.* (2014) with slight modifications. Sample containing 5 g of potato flesh was extracted with 5 ml of 8% acetic acid, sonicated for half an hour and centrifuged for 10 min at 10000rpm. Supernatant was collected, residue was re-extracted with 5 ml of 8% acetic acid. Supernatants were pooled and volume was raised to 12.5 ml with 8% acetic acid and filtered through 0.2 µm PVDF filter before HPLC analysis. Ascorbic acid was analyzed using isocratic mode on HPLC. Mobile phase was prepared by using glacial acetic acid, milliQ water and HPLC grade methanol in 1:69:30 ratio and 10 g sodium chloride was dissolved in mobile phase. The flow rate was kept 1 ml/min, injection volume was 20µl and wavelength used was 273nm. Stock ascorbic acid standard was prepared at 1 mg/ ml mobile phase concentration.

Data analysis

A total of 20 traits of agronomic, physio-biochemical and NUE variables were measured from at least three plants per replication in total two replications. The pooled analysis of variance (ANOVA) for randomised block design was used to analyse two years data of 118 genotypes using the OPSTAT software using the Fisher's test ($p < 0.05$) (Sheoran *et al.* 1998). The homogeneity of variance was tested by the Bartlett's Chi-square test. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed using the XLSTAT (version 2020.4.1) (Addinsoft, 2020). PCA was analysed based on the Pearson's correlation and AHC was estimated based on the Euclidean distance and Ward's Agglomeration method. Comparison of means was done using the Tukey's test ($p < 0.05$).

RESULTS

Development of NUE population

A bi-parental population of 116 segregating progenies was generated by crossing potato varieties between Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient). Seedlings were raised and tubers of 116 progenies (F_1C_1: first clonal generation) were multiplied for two years (2015-16 and 2016-17) and thus advanced to F_1C_2 stage. Further, 116 progenies were evaluated for next two years in field trials with two replications with limited N supply (50 kg N ha^{-1}), and thus clones were advanced to F_1C_3 (2017-18) and F_1C_4 (2018-19) generations. Data were measured in 118 genotypes (116 progenies, and 2 parents) for traits such as plant phenotype, biomass, yield and its contributing traits, total chlorophyll (SPAD value), total N content, NUE parameters and biochemical traits (Table 1). Thus, based on these observations and tuber phenotypes such as shape, uniformity, eye depth, cracking etc., out of 116 progenies only three hybrids NUE/15-8, NUE/15-23 and NUE/15-67 were selected for further field evaluations.

Phenotype and yield traits

All 118 genotypes were evaluated for two years under limited N input (50 kg N ha^{-1}) for phenotype and yield contributing traits such as plant height, total leaf area, shoot dry weight, tuber number, tuber yield and tuber dry matter. Significant differences ($p < 0.05$) were observed in the genotypes for these traits, and effect of years was non-significant in all except tuber number (Table 1). Plant height per plant varied between 13.68 cm (NUE/15-10) to 39.54 cm (NUE/15-14) and total leaf area ranged from 793.94 cm^2 (NUE/15-10) to 4336.56 cm^2 (NUE/15-99). Similarly, shoot dry weight per plant ranged between 11.73 g (NUE/15-103) to 80.57 g (NUE/15-8). Importantly, the highest tuber yield per plant was observed in NUE/15-23 (660.63 g) followed by NUE/15-8 (589.74 g) and NUE/15-67 (566.72 g), whereas the lowest yield was recorded in NUE/15-10 (74.38 g) followed by NUE/15-106 (76.38 g). Tuber number per plant varied between 2.63 (NUE/15-10) to 22.50 (NUE/15-120). Field trials of the selected advance hybrids (NUE/15-8, NUE/15-23, and NUE/15-67) for

Table 1. Mean values of two years data on agronomic, physiological and nitrogen use efficiency (NUE) traits of 116 segregating progenies and their parents (Kufri Jyoti Kufri Gaurav).

SN	Genotype	PH	TLA	SDW	TN	TY	TDM	Chl	Plant N	AgNUE	NUE	NUpE	NUtE	HI	NHI	AA	TSP	RS	Su	TSS	AsA
1	NUE/15-1	26.72	1831.19	22.13	10.31	506.88	22.72	40.43	3.28	10.14	2.75	0.066	41.88	0.84	0.76	210.44	2.33	233.69	114.13	5.96	36.84
2	NUE/15-2	26.61	1935.94	26.68	9.88	164.38	22.72	39.58	1.84	3.29	1.28	0.037	34.72	0.57	0.47	107.19	2.70	171.75	113.69	5.96	51.16
3	NUE/15-3	29.61	3734.25	18.74	11.25	400.00	25.78	39.63	2.89	8.00	2.43	0.058	41.71	0.84	0.77	183.75	3.12	170.44	117.81	6.91	32.99
4	NUE/15-4	22.68	2153.63	21.98	8.44	420.63	21.78	35.75	2.94	8.41	2.27	0.059	38.73	0.81	0.71	171.75	2.90	251.75	68.69	5.71	42.97
5	NUE/15-7	22.62	1696.75	22.43	9.56	265.00	23.66	35.50	1.95	5.30	1.70	0.039	43.61	0.74	0.60	82.81	1.71	101.75	42.81	3.96	37.17
6	NUE/15-8	30.11	3570.19	80.57	11.93	589.74	21.17	36.80	5.84	11.79	4.11	0.117	35.14	0.61	0.48	70.25	1.54	103.06	68.25	5.91	27.49
7	NUE/15-9	28.72	1460.75	59.44	7.00	242.25	17.78	39.15	3.04	4.85	2.05	0.061	33.75	0.41	0.28	126.25	2.23	272.19	109.13	6.35	67.58
8	NUE/15-10	13.68	793.94	14.96	2.63	74.38	17.89	18.55	0.80	1.49	0.57	0.016	35.45	0.47	0.35	65.38	1.58	192.81	73.69	5.51	24.61
9	NUE/15-11	27.27	2587.31	24.43	14.19	440.75	23.72	39.15	3.07	8.82	2.58	0.061	42.09	0.81	0.73	59.13	2.07	291.31	99.13	8.71	56.47
10	NUE/15-12	27.61	2197.06	75.00	8.88	174.00	16.66	40.83	3.25	3.48	2.08	0.065	31.90	0.26	0.18	131.13	1.58	239.13	63.25	9.06	31.84
11	NUE/15-13	23.78	1629.81	56.57	4.88	264.13	18.66	41.65	3.00	5.28	2.12	0.060	35.36	0.46	0.35	109.56	2.39	199.13	108.25	10.96	55.68
12	NUE/15-14	39.54	2282.44	41.69	7.47	403.59	20.06	34.40	3.31	8.07	2.45	0.066	37.05	0.66	0.54	51.13	2.39	195.38	167.38	6.31	47.59
13	NUE/15-15	26.61	1598.00	26.69	4.44	160.13	19.72	36.33	1.67	3.20	1.17	0.033	34.95	0.54	0.43	57.69	1.36	205.44	73.69	5.41	37.04
14	NUE/15-16	25.89	2726.25	30.64	10.56	295.50	19.38	32.45	2.33	5.91	1.76	0.047	37.65	0.65	0.54	76.25	1.96	205.31	44.13	6.91	35.70
15	NUE/15-17	31.16	2428.25	35.46	11.31	296.63	18.72	40.73	2.48	5.93	1.82	0.050	36.49	0.60	0.49	129.13	2.23	150.88	80.13	5.41	26.21
16	NUE/15-18	27.61	2687.31	29.53	5.88	193.75	20.66	40.63	2.00	3.88	1.39	0.040	34.76	0.58	0.48	94.13	2.88	206.75	88.56	5.41	25.63
17	NUE/15-19	23.78	1819.25	26.38	8.13	159.25	20.89	35.63	1.63	3.19	1.19	0.033	36.60	0.56	0.46	114.13	1.73	215.44	78.25	7.06	37.85
18	NUE/15-20	22.10	1920.13	26.44	5.69	132.13	17.72	38.50	1.37	2.64	1.00	0.027	36.09	0.46	0.34	73.25	1.85	261.31	174.13	6.66	18.30
19	NUE/15-21	25.83	1846.19	36.19	7.19	138.38	21.72	38.88	1.85	2.77	1.32	0.037	35.80	0.45	0.35	172.81	3.34	252.63	187.81	7.01	51.63
20	NUE/15-22	27.72	1760.56	21.89	8.81	267.38	17.78	38.25	1.70	5.35	1.39	0.034	40.74	0.68	0.58	98.25	1.93	190.44	93.25	7.91	35.40
21	NUE/15-23	26.94	2773.69	19.79	10.76	660.63	21.78	37.90	3.18	13.21	3.27	0.064	53.31	0.88	0.75	50.25	0.89	170.00	83.25	7.46	31.87
22	NUE/15-24	26.27	3034.94	22.94	3.94	179.63	16.66	38.93	1.46	3.59	1.06	0.029	36.47	0.57	0.42	173.69	1.87	225.44	93.25	7.87	65.23
23	NUE/15-25	29.16	2356.38	29.73	4.25	193.00	16.72	22.10	1.55	3.86	1.24	0.031	39.98	0.52	0.38	108.69	2.78	211.31	108.25	7.16	27.86
24	NUE/15-26	38.10	1140.94	44.09	7.45	345.21	17.66	39.60	2.85	6.90	2.10	0.057	36.92	0.58	0.46	37.81	1.57	221.31	184.13	7.16	36.97
25	NUE/15-27	26.21	1770.19	41.54	12.56	351.50	18.72	38.90	2.54	7.03	2.14	0.051	42.12	0.61	0.44	33.69	1.50	201.75	180.38	7.06	30.56
26	NUE/15-28	25.61	1887.50	18.04	10.31	442.63	20.83	38.65	2.52	8.85	2.20	0.050	43.32	0.83	0.74	92.81	1.87	231.75	128.69	7.16	40.98
27	NUE/15-29	22.72	1257.38	36.13	14.75	460.00	25.72	41.48	3.71	9.20	3.09	0.074	42.12	0.77	0.69	50.69	2.47	181.31	127.81	7.26	30.10
28	NUE/15-30	31.10	2864.13	21.70	7.79	423.70	20.72	37.90	2.73	8.47	2.19	0.055	40.11	0.80	0.73	136.69	2.29	272.25	138.69	4.91	35.54
29	NUE/15-31	29.72	2679.56	24.94	15.56	130.63	19.72	37.53	1.42	2.61	1.01	0.028	35.68	0.51	0.38	166.50	2.50	239.13	139.81	7.16	51.67
30	NUE/15-32	20.68	2074.50	19.03	9.56	256.25	28.78	32.43	2.24	5.13	1.85	0.045	41.28	0.79	0.68	34.13	1.97	155.56	111.13	7.83	18.69

SN	Genotype	PH	TLA	SDW	TN	TY	TDM	Chl	Plant N	AgNUE	NUE	NUpE	NUtE	HI	NHI	AA	TSP	RS	Su	TSS	AsA
31	NUE/15-33	23.72	1850.69	20.26	12.81	331.13	19.78	36.18	2.23	6.62	1.71	0.045	38.53	0.76	0.66	148.69	1.98	185.13	158.25	5.26	41.43
32	NUE/15-34	20.66	2783.13	24.57	9.56	276.00	22.66	38.48	2.17	5.52	1.74	0.043	40.24	0.72	0.60	75.38	2.08	202.25	134.56	6.26	42.97
33	NUE/15-35	22.72	2364.88	48.00	10.50	321.75	18.78	39.43	3.07	6.44	2.17	0.061	35.43	0.56	0.41	148.69	1.67	209.13	113.25	7.66	16.10
34	NUE/15-36	25.66	2655.75	38.01	13.63	251.38	17.89	39.40	2.61	5.03	1.66	0.052	31.94	0.54	0.47	64.94	2.05	239.13	140.69	6.46	27.81
35	NUE/15-37	23.78	1938.00	16.26	6.00	181.13	23.89	37.95	1.48	3.62	1.19	0.030	40.07	0.72	0.60	79.13	1.41	212.25	157.38	7.96	20.73
36	NUE/15-38	21.66	3359.44	37.74	5.13	252.38	15.83	38.55	2.20	5.05	1.55	0.044	35.36	0.51	0.38	353.94	1.76	212.69	87.81	6.96	24.21
37	NUE/15-39	32.89	2052.25	18.23	7.19	475.31	23.78	39.60	2.94	9.51	2.63	0.059	45.02	0.86	0.76	241.31	1.97	188.69	106.50	9.87	16.28
38	NUE/15-40	27.27	4063.13	23.28	11.56	202.25	23.89	36.80	1.90	4.05	1.43	0.038	37.59	0.65	0.52	167.19	1.92	155.13	82.38	8.27	32.33
39	NUE/15-42	34.55	1773.75	36.26	9.19	236.63	18.72	38.18	2.20	4.73	1.61	0.044	36.76	0.55	0.41	143.88	1.58	223.69	110.25	6.96	31.07
40	NUE/15-43	24.66	2185.75	32.13	9.00	293.50	20.78	36.35	2.38	5.87	1.86	0.048	39.19	0.65	0.52	254.50	1.13	237.81	145.13	4.56	21.65
41	NUE/15-44	26.66	1926.56	17.00	14.50	397.13	23.89	38.30	1.60	7.94	2.24	0.032	69.73	0.85	0.65	160.44	1.81	217.25	145.69	7.11	12.99
42	NUE/15-45	38.66	1026.19	59.54	8.75	178.88	19.78	43.60	2.64	3.58	1.90	0.053	36.24	0.37	0.27	167.44	2.20	216.69	121.13	5.26	5.33
43	NUE/15-46	23.72	2000.50	23.33	16.75	439.38	26.66	40.58	3.32	8.79	2.81	0.066	42.46	0.83	0.75	40.63	1.89	217.81	123.69	7.21	7.88
44	NUE/15-47	28.72	2312.38	24.70	8.62	528.33	29.89	43.60	4.36	10.57	3.65	0.087	41.90	0.86	0.80	140.88	2.79	196.25	106.94	4.51	64.24
45	NUE/15-48	23.78	2268.75	19.07	9.44	452.38	20.83	36.40	1.68	9.05	2.26	0.034	66.88	0.83	0.61	66.25	2.88	205.50	122.81	7.11	48.10
46	NUE/15-49	20.17	2121.00	21.05	8.31	125.63	18.78	39.28	1.23	2.51	0.89	0.025	36.18	0.53	0.39	118.69	3.08	132.81	74.56	6.66	71.79
47	NUE/15-50	28.66	2694.56	25.34	9.58	552.97	21.89	39.25	3.42	11.06	2.93	0.068	42.81	0.83	0.73	309.13	2.70	150.69	93.25	6.36	30.65
48	NUE/15-51	24.49	2996.56	17.72	8.75	241.75	16.61	37.45	1.49	4.84	1.16	0.030	38.71	0.67	0.55	40.25	1.21	223.94	109.13	3.87	9.43
49	NUE/15-52	31.89	1265.69	24.33	8.38	227.88	19.66	39.68	1.79	4.56	1.38	0.036	38.32	0.64	0.51	74.56	1.68	146.81	69.13	6.87	25.67
50	NUE/15-53	27.72	1600.94	22.19	10.50	281.25	20.66	37.83	2.05	5.63	1.61	0.041	39.08	0.70	0.60	56.94	1.48	168.69	82.81	3.91	21.07
51	NUE/15-54	30.72	1688.25	30.01	8.00	252.88	18.55	38.73	2.02	5.06	1.54	0.040	38.14	0.61	0.49	86.50	1.82	204.56	114.13	5.67	43.21
52	NUE/15-55	24.78	2501.56	22.86	9.69	347.25	19.66	34.40	2.25	6.95	1.82	0.045	40.62	0.75	0.63	225.56	1.53	119.56	82.38	4.96	28.27
53	NUE/15-56	34.21	1367.63	22.76	10.81	274.38	26.10	38.23	2.23	5.49	1.89	0.045	42.68	0.76	0.63	68.25	3.09	200.69	106.94	9.06	41.76
54	NUE/15-57	24.89	2509.81	22.16	14.31	423.38	21.66	37.58	2.63	8.47	2.27	0.053	42.76	0.80	0.69	73.69	1.87	207.69	129.81	7.81	30.36
55	NUE/15-58	27.27	3010.00	24.74	13.75	296.25	23.44	36.45	2.35	5.93	1.89	0.047	40.15	0.72	0.62	217.38	2.10	186.94	93.25	6.61	34.22
56	NUE/15-59	26.27	2204.06	20.38	7.69	190.88	20.66	38.48	1.52	3.82	1.19	0.030	39.20	0.66	0.54	97.38	2.07	173.69	106.50	7.31	33.16
57	NUE/15-60	23.89	1677.75	20.77	10.56	393.63	23.89	38.88	2.65	7.87	2.30	0.053	43.25	0.82	0.73	45.63	2.66	196.25	118.25	7.41	36.72
58	NUE/15-61	24.72	2219.88	22.78	13.69	374.25	20.49	40.38	2.38	7.49	1.99	0.048	41.60	0.77	0.68	252.63	2.78	222.81	97.81	8.46	73.44
59	NUE/15-62	22.20	2475.44	19.84	11.00	471.75	21.44	36.98	2.71	9.44	2.42	0.054	44.72	0.84	0.75	38.25	2.77	178.25	90.69	8.27	55.09
60	NUE/15-63	29.16	2461.19	17.51	10.39	497.50	23.49	41.85	3.10	9.95	2.69	0.062	43.55	0.87	0.80	149.56	2.59	215.38	124.13	7.51	42.91
61	NUE/15-64	27.55	1928.50	25.09	7.88	316.25	17.72	40.50	1.99	6.33	1.62	0.040	40.61	0.69	0.57	95.63	2.50	105.81	43.69	7.16	51.57
62	NUE/15-66	20.79	2715.50	59.55	8.56	276.63	17.55	35.55	3.03	5.53	2.16	0.061	35.66	0.45	0.32	148.25	1.51	199.13	296.69	7.21	37.34

SN	Genotype	PH	TLA	SDW	TN	TY	TDM	Chl	Plant N	AgNUE	NUE	NUpE	NUtE	HI	NHI	AA	TSP	RS	Su	TSS	AsA
63	NUE/15-67	21.72	1507.44	19.26	8.93	566.72	18.61	43.53	3.08	11.33	2.49	0.062	40.45	0.85	0.78	93.25	0.96	198.81	89.81	8.56	23.16
64	NUE/15-68	33.78	1977.25	42.96	15.31	425.50	17.49	40.83	3.03	8.51	2.34	0.061	38.16	0.62	0.49	86.94	1.49	159.56	64.56	5.66	32.99
65	NUE/15-69	22.72	1120.75	23.05	15.75	494.88	24.49	39.85	3.64	9.90	2.89	0.073	40.15	0.84	0.67	223.06	1.76	167.81	43.25	5.76	38.68
66	NUE/15-70	29.16	1876.31	29.54	13.75	301.75	18.55	40.45	2.22	6.04	1.71	0.044	38.67	0.65	0.51	77.38	1.66	200.44	67.13	3.61	17.88
67	NUE/15-71	28.21	2191.44	32.16	6.88	259.38	18.78	42.75	2.22	5.19	1.62	0.044	36.36	0.60	0.47	200.44	1.68	220.44	209.56	8.61	46.82
68	NUE/15-73	21.16	1209.81	35.71	8.69	298.13	20.66	39.25	2.52	5.96	1.94	0.050	38.09	0.62	0.49	39.13	2.27	248.69	169.56	7.91	34.13
69	NUE/15-74	29.21	2484.00	22.55	3.31	101.25	17.72	43.13	1.18	2.03	0.81	0.024	34.13	0.42	0.30	79.13	2.60	241.31	80.25	10.96	29.40
70	NUE/15-75	30.72	1948.94	16.95	13.06	283.50	17.78	44.93	1.69	5.67	1.35	0.034	39.74	0.75	0.64	90.44	1.26	158.25	78.81	5.67	33.53
71	NUE/15-76	28.72	2234.94	28.38	14.00	382.75	15.66	41.90	1.58	7.66	1.76	0.032	55.12	0.67	0.41	212.19	2.29	150.88	67.50	7.91	44.17
72	NUE/15-77	24.72	2632.13	37.88	6.88	225.38	17.89	43.93	2.17	4.51	1.56	0.043	36.12	0.51	0.39	84.56	1.18	168.25	106.50	7.16	29.25
73	NUE/15-78	16.28	2450.56	44.25	7.13	350.75	12.78	39.93	2.49	7.02	1.78	0.050	35.76	0.50	0.38	55.13	1.95	259.13	93.25	9.83	43.92
74	NUE/15-79	34.78	3556.06	21.66	7.13	231.75	22.16	39.28	1.80	4.64	1.45	0.036	39.05	0.65	0.54	34.25	1.89	150.44	97.81	6.11	20.55
75	NUE/15-80	23.72	1779.75	25.14	9.88	200.38	22.83	38.73	1.58	4.01	1.42	0.032	44.80	0.65	0.48	44.56	2.94	260.00	118.69	7.71	33.01
76	NUE/15-81	25.61	1423.44	29.39	7.19	150.13	23.66	38.73	1.63	3.00	1.30	0.033	39.99	0.55	0.39	88.25	1.61	101.31	60.25	6.81	27.58
77	NUE/15-82	27.55	2418.69	31.70	7.25	186.88	23.78	39.08	2.01	3.74	1.52	0.040	38.14	0.58	0.44	151.31	1.91	168.25	74.13	4.71	50.81
78	NUE/15-83	32.21	2203.69	25.34	6.19	231.88	17.72	39.30	1.74	4.64	1.33	0.035	38.11	0.62	0.48	144.56	1.83	277.81	124.56	8.26	40.58
79	NUE/15-84	21.78	1074.13	26.89	10.94	380.50	17.66	41.50	2.06	7.61	1.87	0.041	44.56	0.69	0.52	129.13	1.50	92.81	50.25	7.83	22.59
80	NUE/15-85	27.72	989.44	22.28	14.69	346.63	24.61	44.03	2.52	6.93	2.15	0.050	42.59	0.79	0.69	76.06	2.06	208.69	60.25	8.76	42.61
81	NUE/15-86	25.66	1217.63	23.80	8.13	191.13	18.66	45.18	1.57	3.82	1.19	0.031	38.03	0.60	0.46	38.69	1.77	246.94	89.81	8.81	28.41
82	NUE/15-87	21.78	1194.94	25.68	6.69	261.00	17.78	38.88	1.86	5.22	1.44	0.037	38.73	0.65	0.52	175.44	1.92	246.94	140.69	9.78	54.10
83	NUE/15-88	27.78	2788.56	44.49	7.53	414.48	23.72	39.78	3.64	8.29	2.86	0.073	39.53	0.69	0.57	129.13	1.30	106.94	39.38	5.46	7.77
84	NUE/15-89	20.83	2354.50	27.00	16.38	433.00	19.78	40.00	2.31	8.66	2.25	0.046	49.33	0.76	0.61	132.81	1.44	210.88	335.31	5.36	28.93
85	NUE/15-90	23.72	2189.06	23.86	10.16	540.47	20.83	39.05	3.24	10.81	2.73	0.065	42.09	0.83	0.74	137.38	1.21	189.56	122.81	7.28	12.52
86	NUE/15-91	28.72	3698.63	30.12	8.44	494.01	18.78	42.53	3.02	9.88	2.46	0.060	40.94	0.75	0.64	68.13	2.04	259.56	138.25	11.59	48.47
87	NUE/15-92	32.78	1718.44	21.77	10.44	85.75	16.78	38.75	1.06	1.72	0.72	0.021	34.16	0.40	0.28	118.25	1.24	300.88	128.69	9.21	47.97
88	NUE/15-93	31.78	3240.00	17.43	20.81	535.63	24.44	40.50	3.24	10.71	2.97	0.065	45.83	0.88	0.81	136.31	1.51	164.13	60.25	5.71	28.04
89	NUE/15-94	25.72	2088.13	26.82	6.13	221.63	16.89	44.68	1.70	4.43	1.28	0.034	37.62	0.58	0.44	221.75	2.07	198.25	99.81	7.96	28.39
90	NUE/15-95	18.28	2221.63	33.72	11.69	288.50	16.27	37.60	2.22	5.77	1.62	0.044	36.53	0.58	0.43	60.44	1.69	96.94	42.81	5.71	21.68
91	NUE/15-96	22.83	2529.56	17.56	14.88	451.88	23.21	34.60	1.73	9.04	2.44	0.035	70.14	0.85	0.68	47.31	2.12	248.69	129.81	4.11	29.22
92	NUE/15-97	23.94	1727.94	17.53	16.38	568.75	19.44	38.80	3.02	11.38	2.55	0.060	42.12	0.86	0.79	169.56	1.37	208.69	494.19	5.51	24.71
93	NUE/15-98	28.55	2115.63	21.18	12.06	323.50	18.56	38.05	2.01	6.47	1.63	0.040	40.19	0.73	0.61	53.38	1.92	137.81	74.13	5.96	28.61
94	NUE/15-99	25.33	4336.56	32.05	8.13	269.38	20.66	37.80	1.64	5.39	1.75	0.033	53.52	0.64	0.37	129.56	1.92	207.81	90.69	8.16	35.61

SN	Genotype	PH	TLA	SDW	TN	TY	TDM	Chl	Plant N	AgNUE	NUE	NUpE	NUtE	HI	NHI	AA	TSP	RS	Su	TSS	AsA
95	NUE/15-100	17.61	2208.56	50.00	12.88	217.50	17.18	42.13	2.58	4.35	1.75	0.052	33.98	0.43	0.29	84.13	1.84	229.13	137.81	7.26	33.03
96	NUE/15-101	24.72	2279.81	32.19	16.75	515.88	20.44	19.70	3.94	10.32	2.75	0.079	34.93	0.77	0.71	114.25	1.81	148.69	86.50	3.91	39.11
97	NUE/15-102	20.61	1637.88	24.31	8.63	296.13	21.44	38.18	2.51	5.92	1.76	0.050	35.00	0.72	0.65	130.44	1.63	218.25	142.81	9.87	38.98
98	NUE/15-103	22.66	2221.19	11.73	4.44	100.88	16.91	38.70	0.82	2.02	0.58	0.016	35.26	0.59	0.49	108.25	1.31	262.81	131.06	10.28	53.21
99	NUE/15-104	20.72	1333.25	25.49	16.50	230.00	23.56	23.88	2.29	4.60	1.59	0.046	34.72	0.67	0.60	64.13	1.93	146.94	74.56	6.37	59.18
100	NUE/15-105	29.72	2219.88	21.15	14.31	275.50	26.56	39.38	2.38	5.51	1.89	0.048	39.24	0.77	0.68	87.69	1.81	219.13	306.94	4.87	27.72
101	NUE/15-106	29.21	2021.25	23.59	5.00	76.38	18.23	40.83	1.06	1.53	0.75	0.021	35.37	0.36	0.27	155.13	2.21	263.69	140.63	13.04	58.29
102	NUE/15-107	30.06	2286.56	21.99	14.31	413.13	18.72	42.63	2.21	8.26	1.99	0.044	45.03	0.78	0.64	84.13	1.91	206.50	88.25	8.16	18.66
103	NUE/15-108	21.56	1285.75	21.44	18.19	275.63	21.66	36.70	1.93	5.51	1.63	0.039	41.97	0.71	0.56	38.25	1.43	237.81	54.56	7.11	28.76
104	NUE/15-109	26.72	1742.88	42.18	8.44	176.63	18.44	39.75	2.31	3.53	1.50	0.046	32.73	0.43	0.34	37.19	1.80	240.88	130.69	6.26	27.74
105	NUE/15-110	27.11	3912.19	23.71	7.69	174.88	23.44	44.58	1.46	3.50	1.29	0.029	44.11	0.63	0.47	128.69	3.08	135.44	74.56	6.36	30.65
106	NUE/15-111	27.17	1975.06	13.51	11.88	348.88	22.66	41.08	1.61	6.98	1.85	0.032	56.92	0.85	0.73	36.50	2.05	237.19	100.69	9.00	35.82
107	NUE/15-112	28.06	1194.50	16.33	4.00	328.75	20.06	37.83	1.81	6.58	1.65	0.036	45.17	0.81	0.69	145.44	1.53	197.19	74.13	3.91	38.36
108	NUE/15-114	27.17	1881.38	17.88	14.69	461.75	19.55	39.60	2.14	9.24	2.16	0.043	52.66	0.84	0.70	140.88	1.40	204.06	104.25	6.16	18.32
109	NUE/15-115	23.17	2085.06	26.49	6.00	153.63	22.49	39.73	1.58	3.07	1.22	0.032	38.91	0.56	0.40	113.88	2.06	203.13	70.25	10.76	22.06
110	NUE/15-116	30.72	1786.38	29.48	13.31	370.75	19.72	41.55	2.54	7.42	2.05	0.051	40.35	0.71	0.59	262.19	1.40	168.25	81.06	5.36	30.47
111	NUE/15-117	27.72	3745.00	38.16	13.25	322.38	23.33	38.50	2.81	6.45	2.27	0.056	40.30	0.67	0.51	29.13	2.06	200.44	72.81	8.11	70.73
112	NUE/15-118	22.72	1264.44	75.75	14.63	519.38	21.66	39.85	3.77	10.39	3.76	0.075	52.50	0.62	0.35	74.56	1.50	133.25	59.81	6.91	33.65
113	NUE/15-119	26.11	2200.63	28.45	6.56	266.13	18.56	40.68	1.93	5.32	1.56	0.039	40.31	0.64	0.47	82.81	1.84	151.13	56.94	6.56	50.58
114	NUE/15-120	26.89	2539.44	22.06	22.50	277.38	23.66	36.43	1.55	5.55	1.75	0.031	56.35	0.75	0.51	64.56	2.19	128.69	98.94	10.61	35.39
115	NUE/15-121	28.78	1833.50	20.41	12.38	333.13	21.55	44.38	2.00	6.66	1.84	0.040	45.18	0.76	0.64	137.19	1.53	188.69	100.06	7.67	33.27
116	NUE/15-122	29.72	3345.31	21.34	13.19	360.63	25.66	40.83	1.73	7.21	2.28	0.035	66.05	0.81	0.58	54.13	1.43	238.25	142.81	10.33	22.85
117	Kufri Jyoti	37.78	3477.63	33.14	6.22	341.98	17.50	36.08	2.28	6.84	1.77	0.046	38.74	0.68	0.57	66.94	1.44	146.94	119.13	7.21	34.85
118	Kufri Gaurav	37.49	2468.25	29.57	9.45	458.70	17.44	43.85	3.25	9.17	2.23	0.065	34.20	0.72	0.64	122.38	1.52	258.69	123.25	5.28	20.04
	Mean	26.58	2185.96	28.66	10.17	315.14	20.55	38.70	2.32	6.30	1.89	0.05	40.93	0.67	0.54	112.81	1.94	198.27	111.58	7.07	34.98
LSD (0.05)	Years	0.632 NS	61.042 NS	4.589 NS	0.377	41.224 NS	0.568 NS	0.508	0.152 NS	0.824 NS	0.223 NS	0.003 NS	2.769 NS	0.032 NS	0.049 NS	2.257	0.078	2.343	6.949 NS	0.198	0.752 NS
	Varieties	0.693	121.172	11.622	4.753	136.82	0.479	4.908	0.779	2.736	0.651	0.016	6.62	0.114	0.13	4.879	0.094	3.409	27.348	0.155	3.15
	Years × varieties	0.981	171.363	16.436	6.721	193.493	0.678	6.941	1.102	3.87	0.921	0.022	9.37	0.161	0.183	6.899	0.132	4.821	38.675	0.219	4.455

Traits are abbreviated as SDW: Shoot dry weight (g)/plant; PH: Plant height (cm); TLA: Total leaf area (cm2)/plant; Chl: chlorophyll content (SPAD value), TN: Tuber number/plant; TY: Tuber yield (g)/plant; TDM: Tuber dry matter (%); NUE: Nitrogen use efficiency; AgNUE: Plant total N (g)/plant; NUpE: Nitrogen uptake efficiency; NUtE: Nitrogen utilization efficiency; HI: harvest index; NHI: Nitrogen harvest index; AA: Amino acids (mg/100g fresh weight); TSP: Total soluble proteins (%); RS: Reducing sugars (mg/100g fresh weight); Su: Sucrose (mg/100g fresh weight); TSS: Total soluble sugars (%); AsA: Ascorbic acid (mg/100g fresh weight). Kufri Jyoti and Kufri Gaurav are the control varieties. LSD: Least Significant Difference at $p < 0.05$.

two years (2019-20 and 2020-21) at varied N (0 i.e. without N, 50 and 180 kg N ha^{-1}) indicated good potential under limited N (50 kg N ha^{-1}) supply than the control (Kufri Jyoti). NUE/15-23, and NUE/15-67 hybrids were N fertilizer responsive as shown under high N dose (180 kg ha^{-1}) application having significantly higher yield and tuber number than control varieties. In the without N dose treatment (0 kg N), no significant differences were observed in the genotypes including controls (Table 2 and Fig. 1).

Chlorophyll, N content and NUE parameters

Significant differences ($p < 0.05$) were observed in the genotypes on per plant basis under field trials for two years under limited N (50 kg N ha^{-1}) for various traits such as total chlorophyll content (SPAD value), plant

Table 2. Means of two years (2019-20 and 2020-21) data for tuber number and yield (t/ha) of selected NUE potato hybrids under different N regimes at 90 days in sub-tropical plain region.

SN	Genotype	N0		N50		N180	
		Tuber number	Tuber yield	Tuber number	Tuber yield	Tuber number	Tuber yield
1.	NUE/15-8	499.43^{bc}	19.13	884.31^{a}	44.92^{a}	751.03^{abd}	37.72^{a}
2.	NUE/15-23	549.93^{d}	18.90	939.73^{b}	46.94^{a}	904.28^{bc}	42.45^{b}
3.	NUE/15-67	479.87^{a}	19.74	748.25^{a}	39.74^{a}	834.17^{b}	42.78^{b}
4.	Kufri Gaurav	426.20^{a}	18.68	684.48^{a}	44.51^{a}	680.76^{a}	35.50^{a}
5.	Kufri Jyoti	398.91^{b}	17.48	618.53^{a}	33.27^{b}	640.13^{a}	33.90^{a}
	Mean	470.87	18.79	775.06	41.87	761.87	38.47
	Years	123.83^{NS}	6.97^{NS}	191.80^{NS}	2.16	82.74^{NS}	4.63^{NS}
$LSD_{0.05}$	Varieties	63.32	3.03^{NS}	236.48	6.80	151.07	7.52
	Years × Varieties	89.55	4.29^{NS}	334.43^{NS}	9.61	216.48	10.64^{NS}

N0: without N; N50: 50 kg N ha^{-1}; N180: 180 kg N ha^{-1}; $LSD_{0.05}$: Least significant difference at $p < 0.05$. NS: Non-significant; Genotypes were compared with the controls (Kufri Garurav and Kufri Jyoti) using the Tukey's test; Different super-script alphabets indicate significant difference at $p < 0.05$ for the particular trait under N treatment.

Fig. 1. *Three NUE advance hybrids (NUE/15-8, NUE/15-23 and NUE/15-67) of potato developed by crossing Kufri Jyoti (N inefficient) and Kufri Gaurav (N efficient) and selected based on agronomic, physiological and NUE traits performance under limited N (50 kg N per ha) supply under field conditions.*

total N content, and NUE variables (AgNUE, NUE, NUpE, NUtE, HI and NHI) (Table 1). Effect of years was non-significant in these traits except chlorophyll content, while effect of genotypes and interactions (years × genotypes) were significant. Chlorophyll content based on the SPAD values ranged between 18.55 (NUE/15-10) to 45.18 (NUE/15-86). Total N content per plant, which included shoots and tubers, was the highest in NUE/15-8 (5.84 g) followed by NUE/15-47 (4.36 g) and NUE/15-101 (3.94 g), and the lowest in NUE/15-10 (0.8 g). Importantly, AgNUE was the highest in NUE/15-23 (13.21) followed by NUE/15-8 (11.79), NUE/15-97 (11.38) and NUE/15-67 (11.33), whereas the lowest in NUE/15-10 (1.49). NUE trait showed a wide range of variation in the genotypes ranging between 0.57 (NUE/15-10) to 4.11 (NUE/15-8). Similarly, NUpE also varied from 0.016 (NUE/15-10) to 0.117 (NUE/15-8), whereas NUtE was the highest in NUE/15-96 (70.14) and the lowest in NUE/15-12 (31.90). HI was recorded in the range of 0.26 (NUE/15-12) to 0.88 (NUE/15-93), whereas NHI varied between 0.18 (NUE/15-12) to 0.81 (NUE/15-93). Overall, hybrids NUE/15-8, NUE/15-23 and NUE/15-67 had high tuber yield and NUE parameters particularly AgNUE and NUE than the controls.

Biochemical parameters

Significant differences ($p < 0.05$) were observed in all genotypes during two years field trials under limited N (50 kg N ha^{-1}) fertilization for biochemical traits such as total free amino acids, total soluble proteins, reducing sugars, sucrose, total soluble sugars and ascorbic acid (Table 1). Effects of years, varieties and interactions (years × varieties) were significant in these traits except effect of year in sucrose content. Total free amino acids content (mg/100 g FW) varied between 29.13 (NUE/15-117) to 353.94 (NUE/15-38), whereas total soluble proteins (%) ranged between 0.89 (NUE/15-23) to 3.34 (NUE/15-21). Reducing sugars content (mg / 100 g FW) was found to be the highest in NUE/15-92 (300.88) and the lowest in NUE/15-84 (92.81), whereas sucrose content (mg / 100 g FW) was found maximum in NUE/15-97 (494.19) and minimum in NUE/15-88 (39.38). Moreover, total soluble sugars content (%) ranged between 3.61 (NUE/15-70) to 13.04 (NUE/15-106). Similarly, ascorbic acid content (mg /100 g FW) varied between 5.33 (NUE/15-45) to 73.44 (NUE/15-61). The hybrids recorded significantly *at par* or slightly higher/lower values in comparison to the controls. Importantly, reducing sugars were also observed lower in all three hybrids than the best control (Kufri Gaurav), which indicates better processing attributes in these hybrids. Collectively, the selected advance hybrids NUE/15-8, NUE/15-23 and NUE/15-67 had low or moderate levels of these biochemical traits.

Cluster, PCA and correlation analyses

Cluster, PCA and correlation analyses were performed in all 118 genotypes. Agglomerative hierarchical cluster analysis based on the Euclidean distance and Ward's method classified them into two main groups I and II consisted of 42 and 76 genotypes, respectively (Fig. 2). PCA was carried out based on correlation method where first two components accounted for 44.33 % of total variability (F1: 32.56% and F2: 13.77%) (Fig. 3). Interestingly, selected hybrids NUE/15-8, NUE/15-23 and NUE/15-67 were clustered together in group I and they had positive factor scores in first (F1) PCA component. The main contributing traits in F1 PCA component were tuber yield (14.84%) and AgNUE (14.84%) followed by NUE (14.04%), HI (10.62 %) and NHI (10.24%), whereas in second (F2) PCA component traits were shoot dry weight (30.72%) followed by plant N (13.18%), NUpE (13.18%), NUtE (11.16%),

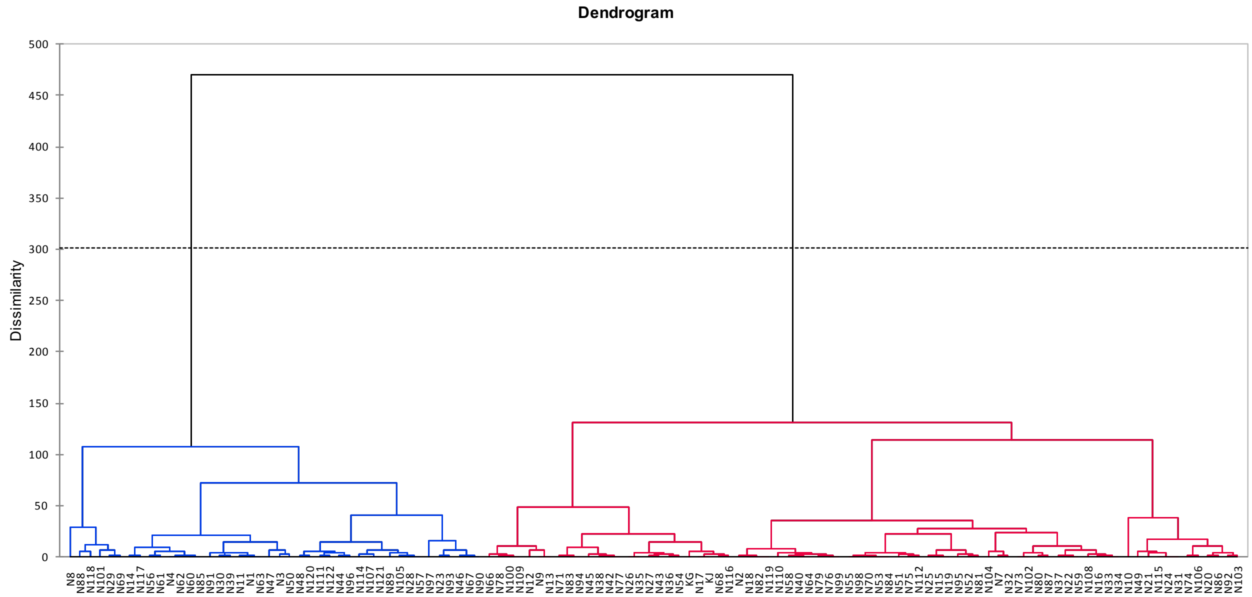

Fig. 2. *Agglomerative hierarchical clustering (AHC) analysis based on the Euclidean distance showing variation in 118 genotypes (116 progenies and 2 parents) for all 20 traits evaluated under field conditions for two years with limited N (50 kg N per ha) supply. Due to space limitation, N indicates 'NUE/15-' in the dendrogram.*

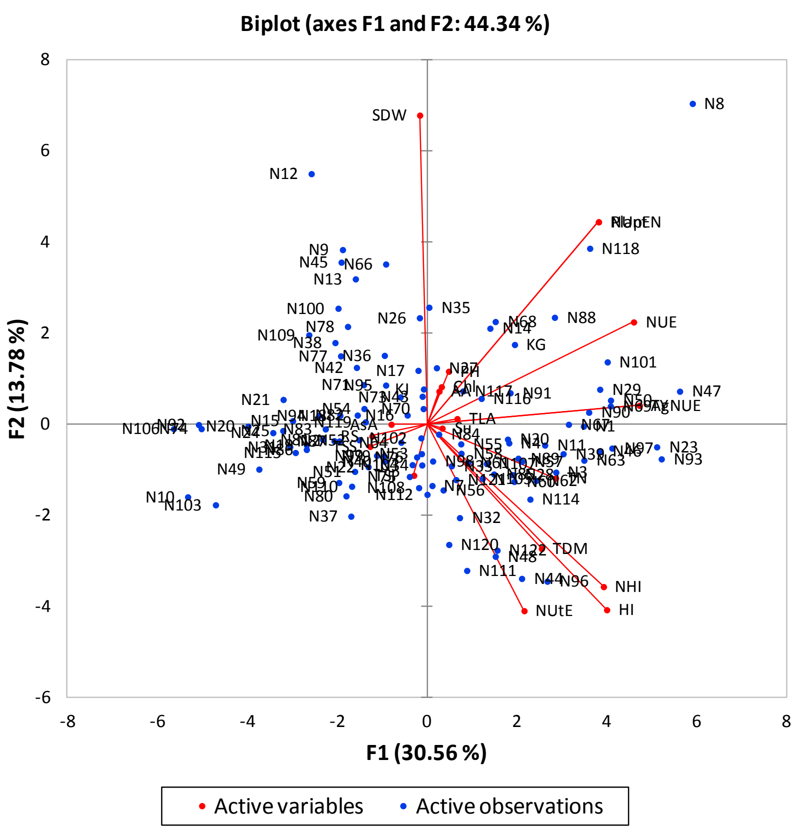

Fig. 3. *Correlation type principal component analysis (PCA) analysis showing distribution of 20 traits and 118 genotypes in a biplot accounting total of 44.34 % variability in the two main principal components.*

Table 3. Correlation coefficient analysis in agronomic, physiological and nitrogen use efficiency (NUE) traits of NUE breeding population.

Traits	PH	TLA	SDW	TN	TY	TDM	Chl	Plant N	AgNUE	NUE	NUpE	NUtE	HI	NHI	AA	TSP	RS	Su	TSS	AsA
PH	**1**	0.155	0.079	-0.022	0.074	0.017	**0.258**	0.121	0.074	0.093	0.121	-0.030	0.014	0.040	0.057	0.032	0.030	-0.023	-0.060	-0.051
TLA	0.155	**1**	0.014	0.011	0.114	0.070	0.027	0.090	0.114	0.116	0.090	0.106	0.087	0.057	0.112	0.106	-0.071	-0.017	0.048	0.016
SDW	0.079	0.014	**1**	-0.070	-0.010	**-0.275**	0.074	**0.503**	-0.010	**0.297**	**0.503**	**-0.318**	**-0.575**	**-0.577**	-0.021	-0.082	-0.061	-0.015	-0.008	-0.001
TN	-0.022	0.011	-0.070	**1**	**0.471**	**0.347**	0.001	**0.308**	**0.471**	**0.454**	**0.308**	**0.384**	**0.466**	**0.411**	-0.085	-0.091	-0.167	0.090	-0.159	-0.103
TY	0.074	0.114	-0.010	**0.471**	**1**	**0.273**	0.086	**0.744**	**1.000**	**0.904**	**0.744**	**0.431**	**0.753**	**0.733**	0.079	-0.112	-0.169	0.083	-0.172	-0.124
TDM	0.017	0.070	**-0.275**	**0.347**	**0.273**	**1**	-0.028	**0.259**	**0.273**	**0.419**	**0.259**	**0.357**	**0.565**	**0.550**	-0.079	**0.266**	-0.167	-0.010	-0.088	-0.037
Chl	**0.258**	0.027	0.074	0.001	0.086	-0.028	**1**	0.067	0.086	0.097	0.067	0.044	0.016	0.000	0.111	0.001	0.118	0.030	**0.241**	-0.037
Plant N	0.121	0.090	**0.503**	**0.308**	**0.744**	**0.259**	0.067	**1**	**0.744**	**0.900**	**1.000**	-0.094	**0.294**	**0.358**	0.078	-0.050	-0.160	0.041	**-0.203**	-0.054
AgNUE	0.074	0.114	-0.010	**0.471**	**1.000**	**0.273**	0.086	**0.744**	**1**	**0.904**	**0.744**	**0.431**	**0.753**	**0.733**	0.079	-0.112	-0.169	0.083	-0.172	-0.124
NUE	0.093	0.116	**0.297**	**0.454**	**0.904**	**0.419**	0.097	**0.900**	**0.904**	**1**	**0.900**	**0.333**	**0.557**	**0.541**	0.047	-0.038	**-0.185**	0.051	-0.167	-0.100
NUpE	0.121	0.090	**0.503**	**0.308**	**0.744**	**0.259**	0.067	**1.000**	**0.744**	**0.900**	**1**	-0.094	**0.294**	**0.358**	0.078	-0.050	-0.160	0.041	**-0.203**	-0.054
NUtE	-0.030	0.106	**-0.318**	**0.384**	**0.431**	**0.357**	0.044	-0.094	**0.431**	**0.333**	-0.094	**1**	**0.584**	**0.396**	-0.077	0.028	-0.072	0.022	0.045	-0.132
HI	0.014	0.087	**-0.575**	**0.466**	**0.753**	**0.565**	0.016	**0.294**	**0.753**	**0.557**	**0.294**	**0.584**	**1**	**0.963**	0.021	0.004	-0.178	0.041	-0.172	-0.104
NHI	0.040	0.057	**-0.577**	**0.411**	**0.733**	**0.550**	0.000	**0.358**	**0.733**	**0.541**	**0.358**	**0.396**	**0.963**	**1**	0.030	0.019	-0.118	0.077	**-0.198**	-0.087
AA	0.057	0.112	-0.021	-0.085	0.079	-0.079	0.111	0.078	0.079	0.047	0.078	-0.077	0.021	0.030	**1**	0.078	-0.009	0.056	-0.061	0.055
TSP	0.032	0.106	-0.082	-0.091	-0.112	**0.266**	0.001	-0.050	-0.112	-0.038	-0.050	0.028	0.004	0.019	0.078	**1**	0.078	-0.063	0.123	**0.418**
RS	0.030	-0.071	-0.061	-0.167	-0.169	-0.167	0.118	-0.160	-0.169	**-0.185**	-0.160	-0.072	-0.178	-0.118	-0.009	0.078	**1**	**0.362**	**0.306**	0.156
Su	-0.023	-0.017	-0.015	0.090	0.083	-0.010	0.030	0.041	0.083	0.051	0.041	0.022	0.041	0.077	0.056	-0.063	**0.362**	**1**	0.001	-0.040
TSS	-0.060	0.048	-0.008	-0.159	-0.172	-0.088	**0.241**	**-0.203**	-0.172	-0.167	**-0.203**	0.045	-0.172	**-0.198**	-0.061	0.123	**0.306**	0.001	**1**	**0.243**
AsA	-0.051	0.016	-0.001	-0.103	-0.124	-0.037	-0.037	-0.054	-0.124	-0.100	-0.054	-0.132	-0.104	-0.087	0.055	**0.418**	0.156	-0.040	0.243	1

Values in bold are different from 0 with a significance level alpha=0.05

Traits are abbreviated as SDW: Shoot dry weight (g)/plant; PH: Plant height (cm); TLA: Total leaf area (cm^2)/plant; SPAD: SPAD value of chlorophyll measurement, TN: Tuber number/plant; TY: Tuber yield (g)/plant; TDM: Tuber dry matter (%); NUE: Nitrogen use efficiency; AgNUE: Plant total N (g)/plant; NUpE: Nitrogen uptake efficiency; NUtE: Nitrogen utilization efficiency; HI: harvest index; NHI: Nitrogen harvest index; AA: Amino acids (mg/100g fresh weight); TSP: Total soluble proteins (mg/100 mg); RS: Reducing sugars (mg/100g fresh weight); Su: Sucrose (mg/100g fresh weight); TSS: Total soluble sugars (%); AsA: Ascorbic acid (mg/100g fresh weight)

NHI (8.46%) and tuber dry matter (4.9%). Similarly, several genotypes had variable contributions in different PCA components. The Pearson's correlation analysis showed significant and non-significant correlations in 20 traits. Importantly, significant and high positive correlations were observed in tuber traits (tuber number, tuber yield and tuber dry matter) with NUE variables (plant N, AgNUE, NUE, NUpE, NUtE, HI and NHI) (Table 3). Shoot dry weight was significantly and positively correlated with plant N, NUE and NUpE; whereas significantly negatively correlated with NUtE, HI and NHI. In biochemical traits, significant and positive correlation was observed in total soluble proteins with tuber dry matter and ascorbic acid; and reducing sugar with sucrose and total soluble sugar. Overall, in our findings, significantly high correlation in tuber traits with plant total N content as well as NUE parameters were observed.

DISCUSSION

In modern agriculture, excessive N fertilizer application is a serious concern while reducing both environmental pollution and production cost for sustainable crop production. Although for many years, improving N fertilization efficiencies have been followed in a wide range of crops applying soil-agronomic management practices. Since NUE of crops is low i.e. below 50% (St. Clair and Lynch, 2010), improving plant NUE is an eco-friendly approach to address these problems. Previous study suggests that yields obtained under recommend N dose is equivalent to high N, hence optimised N fertilizer rate is necessary to ensure both high yields with enhanced NUE (Wang *et al.*, 2020). In this study, out of 116 progenies and selected lines evaluated over the years (2015-2021) under limited N supply based on 20 traits of agronomic, physio-biochemical and NUE, three advance hybrids (NUE/15-8, NUE/15-23, and NUE/15-67) with enhanced NUE and higher yield potential than the control varieties (Kufri Jyoti - the most popular variety in the country, and Kufri Gaurav- an N use efficient variety) were developed.

Significant variations were observed in our genotypes for plant phenotype, agronomic and NUE traits under limited N supply, as reported earlier in potato (Zebarth *et al.*, 2008; Ospina *et al.*, 2014). The selected hybrids (NUE/15-8, NUE/15-23, and NUE/15-67) had significant differences for plant height, total leaf area and shoot dry weight than controls. They also differed significantly in terms of tuber number, tuber yield and tuber dry matter content over controls indicating role of dry matter partitioning and accumulation in tubers (Vos, 1997). For example, NUE/15-23 recorded 52.07% higher yield than the best control Kufri Gaurav, an N-use efficient variety under sub-optimal N condition (Trehan and Singh, 2013). Likewise, NUE/15-8 and NUE/15-23 produced 37.39% and 30.74%, respectively higher tuber yield than in Kufri Gaurav. Moreover, these hybrids had higher marketable and total tuber numbers, and tuber dry matter content than the controls.

Effect of N nutrition on plant growth and tuber yield of potato has been described before (Koch *et al.*, 2020). As mentioned above, we also observed significant variation in the progenies for such traits. Application of high N fertilizers in potato results in more vegetative growth, leaf expansion, canopy development and thus high photosynthetic efficiency (Vos, 1997). Additionally, high N may reduce the proportionate tuber dry matter content than foliage and delays tuberization. N remobilization occurs early under N deficiency and therefore early crop senescence, while in contrast high N delays maturity and so late crop senescence (Zebarth and Rosen, 2007). At later growth stage, high N may increase tuber yield through N remobilization from green leaves to tubers (Vos, 2009). Relationship among the various phenotype and agronomic traits vis-a-vis N application rates has also been established in potato such limited N reduces leaf area, N content and yield, while increasingly N application increases plant biomass and yield (Hu *et al.*, 2014). Our advance hybrids NUE/15-8, NUE/15-23 and NUE/15-67 belonged to the medium maturity group based on total chlorophyll content (SPAD value) and senescence in fields. Interestingly, we observed significant and positive correlation in tuber traits (tuber number, tuber yield and tuber dry matter), plant N and NUE variables (AgNUE, NUE, NUpE, NUtE, HI and NHI) and these three hybrids had significantly higher particularly AgNUE and NUE or *at par* in some cases of NUE variables than controls. Indeed, higher NUE is observed with low N supply than high N (Errebhi et al. 1998, 1999; Zebarth *et al.*, 2004). Moreover, previous study also indicates high regression coefficient for plant dry weight and N content under low N regime (Errebhi *et al.*, 1998). Plant dry weight, yield and plant N accumulation under limited N input could be considered as key selection criteria for enhancing NUE in potato (Vos 1997). Due to shallow rooted attribute, potato crop cannot uptake nutrient from deeper soil layers and hence has lower NUE than deep rooted crops (Iwama, 2008). However, late maturing varieties uptake more nitrogen and has high NUE than early maturing type (Tiemens-Hulscher *et al.*, 2014). Overall, study suggests that tuber yield and NUE variables are the major traits for selection of N use efficient genotype under limited N availability. As a result, advance hybrids NUE/15-8, NUE/15-23, and NUE/15-67 were identified for further field trials.

N fertilization plays key roles in determining tuber quality and yield (Naumann *et al.*, 2020). In this study, significant variations were observed in the progenies for various physio-biochemical traits. However, low to moderate positive correlation was observed with most traits such as ascorbic acid and total soluble proteins, and reducing sugars with sucrose and total soluble sugars. Increasing N supply results in larger tuber size and hence used for processing grade potato production, but simultaneously such tubers are unsuitable for fresh consumption or seed purpose (Zebarth and Rosen, 2007). Amount of sugars determine the tuber quality particularly for processing purpose. In our case, since we conducted our trials with very limited N supply, these biochemical constituents were low to moderate in the selected advance hybrids but varied up to very high levels in other progenies compared to the controls. Based on the amino acids, total soluble proteins, reducing sugars, sucrose, total soluble sugar and ascorbic acid contents the selected advance hybrids could be further evaluated for processing attributes under different N regimes. Taken together, the present study indicates that the hybrids NUE/15-8, NUE/15-23, and NUE/15-67 possess desirable agronomic as well as physio-biochemical traits under limited N input and might have potential for low N input agriculture.

CONCLUSION

Three N use efficient advance hybrids (NUE/15-8, NUE/15-23, and NUE/15-67) based on 20 traits under field trials with limited N (50 kg N ha^{1}) supply were developed. The investigation on plant phenotype, agronomic, tuber traits, physio-biochemical traits and NUE parameters in the progenies and parents (controls) would be useful information while breeding for NUE. Further, more field trials of these advance hybrids would be required particularly under varied N conditions in multiple locations to evaluate performance under different growing conditions. Collectively, these N-use efficient hybrids would be suitable for low-input agriculture for small and marginal farmers of developing countries in obtaining sustainable potato yield.

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