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Short communication

Transferability of sponge gourd EST-SSR markers for genetic diversity assessment of *Luffa* species

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ABSTRACT

Genetic diversity was studied in 47 *Luffa* genotypes with 17 EST-SSR primers, which generated 34 alleles, ranging 1-2 loci per primer. Forty seven genotypes were broadly classified into two different clusters. Cluster I comprised of genotypes DRG-98, Utkal Tripti, DRG-6, Sel-102, Pusa Nasdar, Pusa Nutan, Arka Sujat, DRG-73, DRG-61, DRG-42 and DRG-50, while Cluster II consisted of 36 genotypes, respectively. EST-SSR primer C90830_G3 was found to be highly informative with PIC value of 0.3750. The variability in the species could be credited to introgression and selection as a result of long history of cultivation under varied climatic conditions. The present data provide adequate evidence of the applicability of EST-SSR markers for diversity analyses, cultivar identification and characterization of the *Luffa* germplasm.

Key words: EST-SSR, *Luffa acutangula*, *Lufa hermaphrodita*.

Luffa (2n = 26) is a member of the Cucurbitaceae family and originates from India (Islam *et al*., 3). Out of nine species found in the world, seven *Luffa* species are found in India of which, three species *L. acutangula*, *L. cylindrica*, and *L. hermaphrodita* are edible species. The genus *Luffa* derives its name from the product 'loofah', which is used as bathing sponges, scrubber pads, doormats, pillows, and mattresses and also for cleaning utensils. Apart from important underutilized vegetable cucurbits, *L. acutangula* and *L. hermaphrodita* have great potential as biodiesel crop in future. Ridge gourd is characterised by monoecious sex form and solitary long fruits of 15-30 cm in length with prominent ribbed and rough fruit skin. Satputia has hermaphrodite sex form and produces small fruits in cluster. Its fruits have faint line instead of prominent ridge. As a result of the long history of cultivation of *Luffa* in India under varied climatic conditions, a large numbers of variants have been developed from the cultivars through introgression and selection. These genetic resources harbour valuable genes or adaptation to diverse agro-ecological zones, and resistance to diseases, pests and stress environments (Arora and Nayar, 1). Knowledge of the genetic variation between genotypes of the crop gene pools is an important consideration for the classification and utilization of plant genetic resources in crop improvement. There are many approaches used to quantify the diversity at intra- as well as inter-species level, however, molecular markers are considered to have enormous potential to explore genetic diversity by detecting polymorphisms at DNA level (Singh *et al*., 5). The use of various molecular marker methods, which are independent of environmental conditions. Microsatellites sequences (EST-SSR) are especially suited to distinguish closely related genotypes because of their high degree of variability and they are therefore favoured in population studies (Smith and Devey, 6). Considering the importance of *Luffa*, it is necessary to understand the molecular diversity among *Luffa* genotypes and its subsequent utilization in genetic enhancement of *Luffa* sp.

The experimental material comprised of 47 genotypes of *Luffa* species collected from various parts of India and maintained at Division of Vegetable Science, ICAR-IARI, New Delhi. Healthy leaf samples were collected from young plants. The leaves were bulked from five plants, and DNA was isolated using standard method. The leaves were ground to a fine powder in liquid nitrogen and resuspended in CTAB extraction buffer. The supernatant was extracted with chloroform-isoamyl alcohol (24:1), precipitated in absolute ethanol and the pellet resuspended in Tris-EDTA buffer and purified with 10 mg/ ml RNase. The extracted DNA was quantified using a Nanodrop® spectrophotometer (Thermofisher Scientific), diluted to a concentration of 50 ng/ml in 1 × TE buffer, and the diluted samples were kept at 20°C. A total of 191 EST-SSR primers, derived from *L. cylindrica,* were screened using five samples of different *Luffa* species for optimum amplification and an initial polymorphism survey. Of these, 17 EST-SSR primers were selected for profiling 47 *Luffa* genotypes. PCR was carried out in 25 µl reaction volumes with 100 ng genomic DNA,

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1 U *Taq* polymerase, 1 × *Taq* buffer,0.4 µM primer, 20 µM of dNTPs mix, with sterile distilled water to make up the volume. For PCR cycling - Bioer XP cycler was programmed as follows: initial cycle at 94°C for 5 min., 40 cycles at 94°C for 20 s, 48-65°C (depending on primer used) for 30 s, and 71°C for 1 min., followed by a final extension at 72°C for 5 min. PCR product was resolved using 2% agarose gels in 1 × TBE (Tris-borate EDTA) buffer for 4 h at 120 V and visualized with ethidium bromide. A 200 bp ladder (Fermentas) was used as molecular weight marker.

All amplicons were scored based on presence/ absence to produce a binary matrix (1=presence/ 0=absence). Only clear and strong bands were recorded and used for further analysis. Polymorphic information content of the primer was calculated. Pairwise genetic similarity coefficient between individuals were calculated by shared allele distance using PowerMarker v3.25 software.

Total 191 EST-SSR markers derived from *L. cyclindrica* were tested on five genotypes each of *L. acutangula* and *L. hermaphrodita* to assess the transferability of primers across the species. Out of which, 131 numbers of primers amplified in the tested species. These markers were further used for polymorphic profiling across the species and 17 markers gave satisfactory results. Sum total of 17 SSR could amplify 34 alleles, ranging 1-2 loci per primer pair. A high degree of molecular polymorphism was exhibited by all the markers studied. The polymorphic information content ranged from 0.1124 for C87552_ G3 and 0.3750 for C90830_G3. The major allele frequency, gene diversity, heterozygosity and PIC data is presented in the Table 1. Higher the PIC value more will be the usefulness of primer, hence, primer C90830_G3 was found to be highly informative as more number of genotypes can be differentiated by using this primer. Forty seven genotypes were broadly classified into two different clusters as shown in Fig. 1. Cluster I comprised of genotypes DRG-98, Utkal Tripti, DRG-6, Sel-102, Pusa Nasdar, Pusa Nutan, Arka Sujat, DRG-73, DRG-61, DRG-42 and DRG-50. Cluster II comprised of 36 genotypes belonging to *Luffa hermaphrodita*, respectively. The similarity indices among the genotypes within the cluster II were high, suggesting that the genomes of the genotypes do not differ much from each other. The low level of intra-specific diversity could be due to gene flow between them as they are crossable. The narrow genetic diversity within intra-specific varieties has also been reported in *Luffa*. However, all the genotypes clearly differentiated into two clusters, suggesting their distinct taxonomic identity. The diversity at species level could be attributed to its long history of cultivation under varied climatic conditions leading to introgression and selection. Our findings corroborates the findings of Cruz *et al*. (2) and Marr *et al*. (4). Higher the dissimilarity between the genotypes

Table 1. Polymorphic information content of EST-SSR markers used in the analysis.

Marker	Major allele frequency	Gene diversity	Heterozygosity	PIC
C84961_G1	0.6702	0.4421	0.0213	0.3443
C89766_G1	0.7660	0.3585	0.0000	0.2943
C85516_G2	0.6170	0.4726	0.0000	0.3609
C88424_G1	0.7979	0.3225	0.0213	0.2705
C83178_G1	0.8511	0.2535	0.0000	0.2214
C82204_G1	0.7660	0.3585	0.0000	0.2943
C73141_G1	0.5851	0.4855	0.6170	0.3677
C90830_G3	0.5000	0.5000	0.0213	0.3750
C83880_G2	0.7660	0.3585	0.0000	0.2943
C89529_G2	0.9255	0.1378	0.0213	0.1283
C76892_G1	0.8298	0.2825	0.0000	0.2426
C84593_G2	0.7660	0.3585	0.0000	0.2943
C83264_G3	0.9255	0.1378	0.0213	0.1283
C89405_G3	0.5745	0.4889	0.8511	0.3694
C87552_G3	0.9362	0.1195	0.0000	0.1124
C89455_G1	0.7872	0.3350	0.0000	0.2789
C85233_G2	0.5106	0.4998	0.1277	0.3749

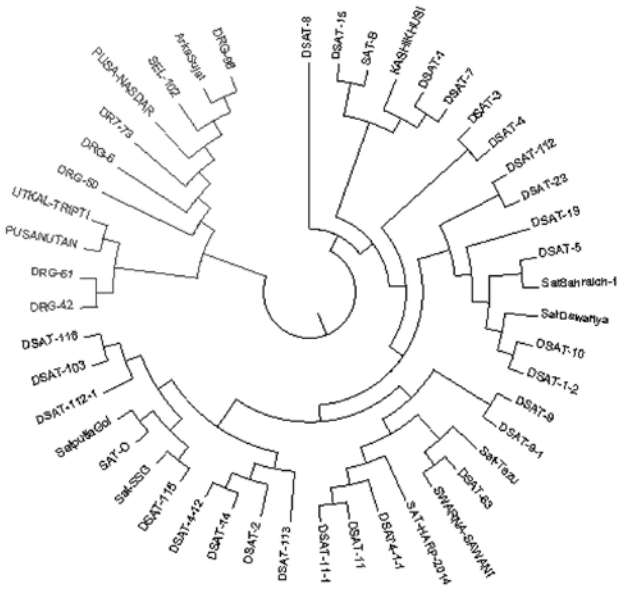

Fig. 1. Cluster diagram of the genotypes of *Luffa* species as obtained from power marker v3.25 software.

better is the scope to include them in a hybridization programme for getting the transgressive segregants. Therefore, to exploit heterosis in *Luffa* the genotypes belonging to different clusters can be used as parent to produce hybrid rather than selecting parents within the cluster. The clustering obtained in this study would be stable even in addition of newer markers and there is less chance of a change in this grouping pattern. The present data provide adequate evidence of the applicability of EST-SSR markers for diversity analyses, cultivar identification and characterization of the *Luffa* germplasm.

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Short communication

Phosphorus efficient potato cultivars for Nilgiris

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ABSTRACT

Field experiments were conducted at Central Potato Research Station, Muthorai, the Nilgiris, Tamil Nadu for three years with seven potato cultivars under four levels of phosphorus application to evaluate their phosphorus use efficiency. Cultivars Kufri Neelima and Kufri Swarna were found more P efficient because of their higher relative biomass production, tuber yield efficiency index, harvest index, agronomic use efficiency (AUE) and P uptake efficiency. These two cultivars produced higher tuber yields under no P and at P_{max} of standard variety. The cultivars Kufri Girdhari, Kufri Jyoti and Kufri Himalini proved P responsive as they responded well to its application and produced very low yields under no P application. Higher root biomass in these two efficient cultivars could be the reason behind their higher P use efficiency in comparison to other cultivars. The two P efficient potato cultivars also happened to be resistant to potato cyst nematode, which are very common and serious problem in Nilgiris. The mechanism to have resistance against PCN, whose cysts emerge only in the presence of root exudates of susceptible potato cultivars, could also have benefitted those cultivars to show more efficiency in native P utilization. Further investigations are required to find out the exact mechanism of P efficiency in these two PCN resistant cultivars.

Key words: Agronomic use efficiency, phosphorus use efficiency, potato, tuber yield efficiency.

Phosphorus use efficiency is generally very low at <30% in potato. Cultivated soils contain good reserves of P and its availability to the plants is seized because of transformations to other forms depending upon soil pH. P is limiting because of its chemistry, *i.e.*, low solubility of phosphates and their rapid transformation to insoluble forms (Smil, 9). Al, Fe, Ca, K, and Mg can all react with fertilizer P and produce relatively insoluble compounds (Smil, 9). Potato is classified as “inefficient responder” to P application (Miyasaka and Habte, 8). Hence, the need to improve P use efficiency is more important in the future due to economic environmental and mineral resource availability pressures.

Lee *et al*. (7) reported that the cultivar adaptation to low-P stress growing conditions depends on various traits, such as mobilization of insoluble phosphates, utilization of limited bioavailable P sources, and P-uptake efficiency. An elite genotype that can adapt to P-limiting growing conditions needs to be excellent in each of the above traits. If such cultivars are identified and their mechanism is known then it becomes easier to breed varieties with higher P efficiency through improved biotechnological tools. Potato is widely grown in Nilgiris with large doses of P application under lateritic soil conditions. If P efficient cultivars are identified and recommended, it can avoid soil build up of P, thereby eutrophication of water bodies. Hence, the present investigation was carried out.

A field experiment was conducted at Central Potato Research Station, Muthorai, the Nilgiris, Tamil Nadu during 2010 to 2012 for three years by planting seven different potato cultivars under four different levels of P (0, 50, 100 and 150 Kg P_2O_5 per hectare) application. The seven cultivars tried were Kufri Swarna, K. Jyoti, K. Neelima, K. Girdhari, K. Shailja, K. Giriraj and K. Himalini, which differ in their maturity periods. The trials were initiated during summer season (April to August) under rainfed conditions as Nilgiris receive good amount of (800 mm) rainfall during South-West monsoon. The plot size adopted was 2.4 × 2.0 m with four rows of potato having 10 plants in each row (at 60 × 20 cm spacing). Standard cultural practices were followed for interculture and harvesting by cutting the haulms 15 day before harvest. The soil type in experimental plot was sandy loam with high available N and P and medium at K. The soils of Nilgiris are rich in P but the availability is very less because of the transformation of P to Fe and Al phosphates.

Per cent emergence of different cultivars under four levels of P application was estimated after one month of planting. At 45 days, plant height, shoot number and number of leaves per plant were recorded by selecting five plants per plot at random. Shoot weight, root length and root biomass were estimated at 90 days after planting. Tuber number and yield

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was estimated in different size grades (<25, 26-50, 51-75 and >75 g) after separation. Tuber yields were recorded net plot wise in all the three years in four different size grades. The total biomass of plants were recorded at 90 days and the tuber yield was at 120 days. Harvest index was calculated in all the varieties at different P levels. P uptake was estimated in different plant parts at 90 days after planting by drawing samples from five plants in each plot. Phosphorus content in tubers was estimated at harvest. Soil samples were analysed for nutrient status using standard procedures before and after the conduct of the experiment. Different nutrient efficiency indices were estimated, *viz.* tuber yield efficiency index, tuber harvest index, agronomic use efficiency (AUE), and phosphorus uptake efficiency (PUE). The pooled data of three years was used to fit quadratic models for yield estimation in seven cultivars and the P_{max} was estimated for standard cv. Kufri Jyoti (Govindakrishnan *et al.*, 4). The yields of different varieties at P_{max} were estimated using the quadratic equations developed.

Plant height, number of shoots per plant and leaf number was significantly affected by P application in potato varieties. The two varieties Kufri Swarna and Kufri Neelima performed better in terms of plant height, number of shoots and leaf number than rest of the cultivars. Efficiency of the above two genotypes for utilization of P could be witnessed from the initial stage itself as the plant growth parameters (Table 1). Potato being a heavy feeder requires higher levels of nutrients from the initial stage itself. Non-availability of required quantities of P might have caused imbalance in many of the treatments leading to reduction in growth and growth parameters. Shortage of phosphate supply was found to increase mainly the ratio of root length per weight of plants (Fist, 2; Jungk *et al*., 6; Trehan and Sharma, 10). The regulating mechanism is reported to be root cell elongation (Anuradha and Narayanan, 1).

The yield produced by K. Neelima and K. Swarna without P application was higher than all other cultivars even at their highest levels of P application except for K. Girdhari at 100 kg P that too was higher than K. Swarna at zero level of P application. This indicates that these two cultivars are highly native P efficient under Nilgiri conditions. The cultivars K. Swarna (31.3 t/ha) and K. Neelima (32.9 t/ha) produced very high yields under no application of P and the cultivars K. Jyoti, K. Himalini and K. Girdhari responded very well to the application of P at different levels (Tables 1, 2 & 3). Efficiency of the above two cultivars in utilization of soil available P could result in increased tuber yield even at zero level of P application under acidic soil conditions of Nilgiris.

This gives an indication about the efficiency of a particular cultivar to yield efficiently under non application and high level of application of a particular nutrient in comparison with other varieties. Tuber yield efficiency index was high in K. Swarna and K. Neelima as they could produce more yields at P deficient conditions. Among the seven cultivars tested K. Shailja and K. Giriraj were the least P efficient (Fig. 1). Cultivars K. Girdhari, K. Himalini and K. Jyoti

Table 1. Growth parameters, yield components and efficiency indices of potato cultivars.

Cultivar	Pl. ht. (cm)	No. of shoots	No. of leaves	Yield/ net plot (kg)	Tuber No./ net plot	P content in tubers (%)	Plant P conc. (%)	P uptake in stems (kg/ ha)	Stem DMP (t/ ha)	Tuber DMP (t/ ha)	AUE
K. Jyoti	22.76	2.58	17.69	4.49	62	0.2868	0.14	2.02	1.44	4.65	150.13
K. Swarna	33.53	3.05	23.12	6.64	65	0.2988	0.10	2.21	2.21	6.91	222.98
K. Girdhari	24.97	2.87	18.31	4.83	65	0.2955	0.13	2.02	1.56	5.03	162.13
K. Shailja	12.47	1.45	10.85	1.16	19	0.2867	0.14	0.51	0.36	1.21	38.90
K. Himalini	22.73	2.41	15.20	3.58	68	0.2965	0.14	1.57	1.12	3.73	120.39
K. Giriraj	18.01	2.26	14.43	2.78	42	0.2977	0.10	0.90	0.90	2.90	93.42
K. Neelima	31.19	2.69	23.03	6.79	80	0.2913	0.12	2.80	2.33	7.07	228.06
$LSD_{0.05}$	2.711	0.31	2.356	0.607	7	0.0046					
P0	20.31	2.26	15.31	3.59	50	0.2755	0.12	1.39	1.16	3.74	120.77
P50	24.99	2.64	18.43	4.59	61	0.2932	0.13	1.99	1.53	4.78	154.19
P100	25.15	2.58	18.59	4.72	60	0.2993	0.13	2.05	1.57	4.92	158.71
P150	24.21	2.40	17.75	4.37	58	0.3052	0.14	2.10	1.50	4.55	146.84
$LSD_{0.05}$	2.050	0.235	1.781	0.462	5	0.0035					

Table 2. Tuber yield per net plot (kg) in different potato cultivars.

Cultivar	P_0	P_{50}	P_{100}	P_{150}
K. Jyoti	3.3783	4.6433	4.8133	5.0367
K. Swarna	6.0200	6.6583	6.7517	7.1100
K. Girdhari	3.4783	5.0267	6.2700	4.5283
K. Shailja	0.9483	1.4167	1.2467	1.0150
K. Himalini	2.5667	4.1450	3.8367	3.7850
K. Giriraj	2.4383	3.1733	3.0183	2.4900
K. Neelima	6.3250	7.0633	7.1317	6.6283

were intermediate and P responsive. This shows that the cvs. K. Neelima and K. Swarna are highly effective in utilizing the native P.

Harvest index represents conversion efficiency of vegetative source to economical part. The cultivars K. Neelima and K. Swarna recorded the highest values for harvest index indicating that they are the most P efficient and the HI increased with increase in P level upto 150 kg per hectare (Fig. 1). That means these two cultivars are more efficient in converting source into economical parts. Cultivars K. Neelima (228) and K. Swarna (222) had higher agronomic use efficiency (AUE) compared to K. Girdhari (162), K. Jyoti (150) and K. Himalini (120), which showed moderate values. The least AUE values were recorded for K. Shailja (38) and K. Giriraj (93) (Table 1). Earlier, Trehan and Sharma (10) reported that Kufri Pukhraj was the most N, P and K efficient cultivar. The P uptake efficiency indices recorded higher values at no P

Table 3. ANOVA for tuber yield in potato.

Source	df	Type III SS	Mean square	F value	Pr>F
Rep (year)	3	2.4639107	0.8213036	0.73	0.5380
Year	2	154.4015512	77.2007756	68.23	<0.0001
Variety	6	591.4661988	98.5776998	87.12	<0.0001
P level	3	32.1779833	10.7259944	9.48	<0.0001

application in K. Neelima and K. Swarna indicating their efficiency to convert native P to available forms. Other genotypes showed a very low uptake efficiency at no application and the values were lower at higher levels of P application (Fig. 1). The variation in phosphorus efficiency of different potato cultivars was due to both their capability to use absorbed P to produce potato tubers (PUE) and to their capacity to take up more P per unit soil. Trehan and Singh (12) reported that K. Pushkar was more P efficient than K. Pukhraj.

Quadratic models were developed for all the cultivars and from them the optimum P dose was estimated (economic optimum). The tuber yield at economic optimum was 37.7 t/ ha in K. Neelima and 35.0 t/ ha in K. Swarna. The cultivars next in order were K. Girdhari (30.9), K. Jyoti (27.4), K. Himalini, K. Giriraj (16.3) and K. Shailja (6.9) (Table 4). The P_{Max} for standard variety K. Jyoti (135 kg/ha) is estimated using the technique developed by Govindakrishnan *et al.* (4). The yields at P_{Max} and at no P also followed similar trend.

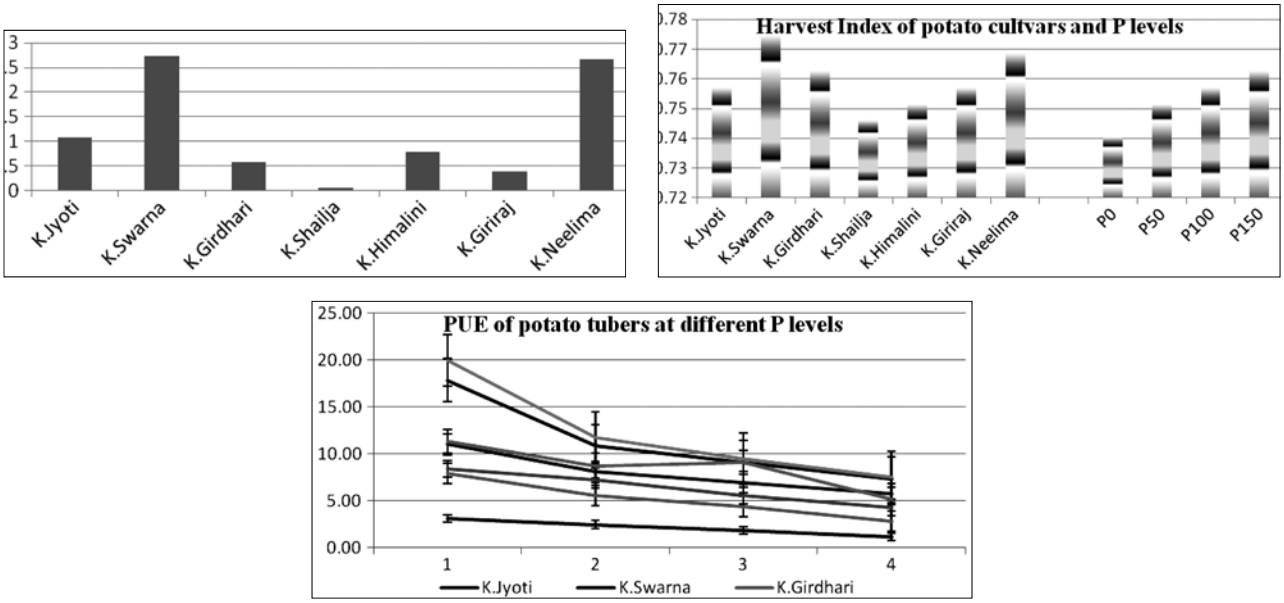

Fig. 1. Tuber yield efficiency, tuber harvest and phosphorus uptake efficiency indices in different potato cultivars.

Table 4. Yield at economic optimum, P_{Max}, no P and dry root biomass of potato cultivars.

Cultivar	Quadratic equation	Econ opt (kg/ ha)	Yd at Econ opt P (t/ ha)	Yd at Max P of std cultivar (t/ ha)	Yd at no P (t/ ha)	Root dry weight (g/ plant)
K. Jyoti	$y = -0.0005x^2 + 0.135x + 17.895$ $R^2 = 0.8935$	132	27.4	27.0	17.9	1.72
K. Swarna	$y = -0.0005x^2 + 0.0884x + 31.14$ $R^2 = 0.7459$	85	35.0	40.2	31.1	3.07
K. Girdhari	$y = -0.0017x^2 + 0.3028x + 17.418$ $R^2 = 0.8539$	88	30.9	26.5	17.4	2.61
K. Shailja	$y = -0.0004x^2 + 0.055x + 5.0894$ $R^2 = 0.6745$	65	6.9	14.2	5.9	1.54
K. Himalini	$y = -0.0008x^2 + 0.1622x + 13.926$ $R^2 = 0.6822$	99	22.1	23.0	13.9	2.52
K. Giriraj	$y = -0.0007x^2 + 0.0987x + 12.834$ $R^2 = 0.4901$	68	16.3	21.9	12.8	1.98
K. Neelima	$y = -0.0006x^2 + 0.1072x + 32.968$ $R^2 = 0.9475$	87	37.7	42.1	32.9	3.14

Econ opt = Economic optimum, Yd = Yield, P opt (kg/ha) = - (cp-b)/2c, Cp = Cost of P fertilizer per kg/ price of potatoes per tonne = (37.5/ 12000) = 0.003125

The root biomass (dry) produced in K. Neelima (3.14 g/ plant) and K. Swarna (3.07 g/ plant) were significantly higher on an average at all the levels of P application substantiating their efficiency in utilizing soil available and applied P resources. Further, these two genotypes are resistant to PCN infection, which makes them maintain healthier roots without any cysts when compared with other cultivars. This could also have been contributed for their better P use efficiency. Lee *et al.* (7) reported that 'Harley Blackwell' and 'Satina' cultivars to show greater P mobilization ability in soils without supplemental P. The ability to uptake more P from soil available level made the cultivars K. Neelima and K. Swarna more efficient in producing better tuber yields in comparison with other inefficient cultivars. Nutrient efficient plants are defined as those plants, which produce higher yields per unit of nutrient, supplied or absorbed than other plants (standards) under similar agro-ecological conditions (Trehan and Singh, 12). The main properties that affect the uptake of nutrients from soil are kinetics of ion absorption by roots, the size of root system and morphological root properties as reported by Jungk and Claassen (6). Gahoonia (3) also reported that phosphate availability could be influenced by root induced changes of soil pH. Investigations are required to confirm the actual reasons for P efficiency.

Different indices were estimated to evaluate their efficiency and it is concluded that among the seven cultivars tested Kufri Neelima and Kufri Swarna are the most P efficient cultivars under Nilgiri conditions where the soils are lateritic with more Fe and Al phosphates. The P efficient cultivars maintained higher vegetative biomass from the initial stages itself and higher root biomass in comparison with others. The higher root volume of these cultivars has contributed for better uptake and thereby enhanced yields even under P deficient (No P) conditions. These cultivars possess resistance to PCN, which is also a positive factor for their higher root activity and there by better utilization of available P. Mineral P resources are dwindling very fast and use of P efficient cultivars is an alternative. Potato tubers are highly correlated with P fertilization. Further studies are required to understand the root morphology and the mechanism of P efficient varieties in utilizing P in a better way for producing more tuber yield. Further, the cultivars K. Girdhari, K. Jyoti and K. Himalini responded greatly to the application of P and proved better P responsive.

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