Evaluation of Mandarin (*Citrus reticulata* Blanco) Cultivars to Expand Production Period in Eastern Hills of Nepal

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Abstracts

Mandarin is a favored fruit of Nepal and mostly grown in hilly terrain. There is good amount of genetic diversity within the country for this crop; however, it has short harvest period. Therefore, an attempt has been made to identify cultivars suitable for extending harvesting period by exploiting 18 accessions collected within countries and introduced from abroad. Two years old trifoliate orange (Poncirus trifoliata) grafted plants were transplanted at 3 m x 3 m spacing in hilly terrace with five replications. The two years data (13th and 14th years after planting) on fruit physical and quality parameters along with yield were considered for the study. Unshiu and Satsuma mandarins (Okitsuwase and, Miyagawawase) have performed well as early season (September) with bigger sized fruit (7-11%) as compared with landraces in NCRP farm at an altitude of 1200 m after 14 years of planting. Similarly, some hybrid accessions (Commune and Nules) and landraces (Banskhark and Khoku Local) have shown potential for mid-season production. However, none of the accessions were good enough to be promoted for late season production. Therefore, research system needs to search for some exotic materials to be promoted as late season production. Murcott and Nagpuri mandarin could be few options for late season production and need to be explored in future. Further, a hybrid cultivar development system including landraces and exotic materials could be another avenue for exploration in the future.

Keywords: *Brim A, dendrogram, harvesting period, maturity, principal component analysis*

Introduction

Hindu Kush Himalayan Region of Nepal, India and Bhutan cultivate same kind of indigenous mandarin genotypes (loose skin; Khasi mandarin) in the hilly slopes (Ghosh, 1993). These genotypes grow well at an altitude of 800-1400 meter with short harvest period during November to January. Due to this short harvesting period, there is market glut and low price for the produce during main season and this leads to import of exotic mandarin from India, Pakistan, Thailand and Australia during off season in Nepal (MoAD, 2017). Therefore, with a view of identifying suitable exotic as well as indigenous cultivars of mandarin for extending production period, a varietal evaluation study was conducted in the farm of National Citrus Research Program (NCRP), Dhankuta, Nepal.

Materials and Methods

Thirteen and fourteen years old mandarin trees of eighteen accessions, which were planted by splice grafting on trifoliate orange *(Poncerous trifoliate)*, were used for varietal evaluation in NCRP farm, Dhakuta Nepal. NCRP farm is located at an altitude of 1200 m with geographical location of 27° 1" north latitude and 87° 18" east longitude. These eighteen cultivars were replicated 5 times (individual plant) and planted at 3 m x 3 m distance in September 2004. The plants were fertilized as per national recommendation of Nepal (NCRP, 2002). Further, the plants were regularly sprayed with recommended pesticides to control major insect pests (leaf minor by Bensyp/Delfin; scale insect by Rogar and petroleum oil) and diseases (Powdery mildew by sulfex/Karathen; sooty mold by Delfin) prevalent in the region as per national guidelines. Fruits were harvested when 50% skin color of the fruits turned

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yellowish/orange. Randomly, five fruits were taken for quality analysis from four sides of a tree. Total soluble solid (TSS) was recorded with hand held refractometer while the Titrable acidity (TA) was analyzed and citric acid amount was derived using titration method. The amount of citric acid in juice was calculated using the following formula:

$$TA\% = \frac{\text{Volume of titrant*} \times \text{Normality of titrant} \times \text{Milli-equivalent weight of citric acid**} \times 100}{\text{Volume of sample*}}$$

*Volume of titrant (5 mL); **Milli-equivalent weight of citric acid (0.064)

Reading was taken twice for each sample and averaged. The BrimA values were calculated as described by Acharya (2017). The formulae for BrimA calculation is as follows:

BrimA1 = {TSS-k(TA)}, where k (5) is a constant factor.

There was no fruiting in Page mandarin in the year 2017. The data were analyzed by using R software (version 3.5) with add on package agricolae (v 1.2-4) while the graphs were drawn by using package corrplot (v 0.1-20) and ggdendro (v 0.1-20).

Results and Discussion

Fruit Physical Parameter

Average weight of ten fruits were considered for this parameter. In the year 2016, the average fruit weight showed significant differences among the tested cultivars while it was non-significant in the year 2017 (Table 1). The pooled analysis from two years data showed highly significant difference on fruit weight. Satsuma Okitsuwase variety produced the biggest fruit (152.67g) followed by Satsuma Mino (142.77 g), Pongan (139g), whereas the Page (63g), Commune and Nules ((73g) were the lightest fruit bearing cultivars. The earlier Unshiu cultivar fruits were 7-11% bigger in size as compared to check cultivar Kokhu local. Similar type of findings on the biggest fruit of Okitsuwase produced in Dailekh district was reported by Chalise and Acharya (2017) and on the lightest fruit was also reported by Neves et al (2018).

The average diameter of five fruits were taken while deriving this parameter. In year 2016, this parameter was highly significant while it was only significant in the year 2017 (Table 1). The average of two years data was highly significant. From average of two year, the bigger diameter of fruit (71 g) was produced by variety Satsuma Okitsu (71 mm) followed by Pongan (66 mm) and Satsuma Mino (66 mm), whereas Page (41 mm), Nules (54 mm) and Commune (56 mm) were the smaller size fruit producing cultivars. Among the land races (Khoku, Banskhark, Sikkime), Khoku local (61 mm) was the bigger diameter of fruit producing variety.

The rind thickness (mm) was derived from average peel thickness of five fruits. It was highly significant for the both tested years as well as pooled analysis of two years data (Table 1). The thinner rind was obtained from Page (1.4 mm) followed by Sikkime Local (1.7 mm) and Miyagawa wase (2.1 mm) while the thicker rind was found on cultivars Fortune (2.7 mm) followed by Pongan (2.7 mm) and Satsuma Mino (2.6 mm). The thinner fruit rind variety are more prone to physical damage while the thicker rind cultivars are difficult to peel and less liked by the consumers. Therefore, a variety with medium thickness rind is considered better due to less physical damage during post-harvest handling and is also liked by consumer because of easiness in peeling.

The number of segment per fruit was found highly significant among tested cultivars for both year and pooled analysis of both year data. (Table 2). The number of segments ranged from 9-12 among the cultivars tested. The highest numbers of segments (12) were recorded in variety Kara and it was at a par with Satsuma Okitsu (12) and Satsuma Mino (12) while the least numbers of segments (9) were recorded in Marisol and Page.

Fruit Quality Parameters

The juice content as expressed in percentage was derived from average of five fruits and it was found highly significant in year 2016 and year 2017 and pooled mean of two years (Table 2). The highest juice percentage (53.1%) was

recorded in variety Satsuma Mino followed by Satsuma Okitsu (51.93%) and Miyagawa wase (49.63%), whereas the Pongan (32.43%) was the least juicy fruit followed by Commune (41.37%) and Oraval (41.7%).

Sweetness of evaluated cultivars measured as total soluble solids (TSS %) was found highly significant in the year 2016, 2017 and followed same pattern for average of two years data (Table 2). The cultivars Fortune and Page (13% TSS) were the sweetest fruit followed by Pongan and Kara (12%). Indigenous land races (Banskhark Local, Khoku Local and Sikkime Local) were also at par with above mentioned cultivars for this trait. The least sweet fruits were obtained from cultivars Miyagawa wase (9%) followed by Okitsu wase (9.2%) and Satsuma Okitsu (9.5%). The TSS % of hybrid cultivars Kara and Nova was similar with the findings from Simon-Grao et al. (2014). The average TSS value of Miyagawawase from this finding was different from the report of Shrestha et al. (2012), while the sweetness value from cultivar Okitsuwase was in agreement with the report of various researchers (Chalise and Acharya, 2017; Paudel and Shrestha, 2002; Shrestha et al. 2012).

Sourness of fruit (citric acid) measured as titratable acidity was recorded in between 0.78 to 2.6% (Table 3). The TA% of the tested cultivars was found highly significant for the year 2016, 2017 and the average of two years followed same pattern. The most sour fruits were produced by cultivars Fortune (2.6%) followed by Kara (2.0%) and Page (1.6%). The less sour fruits were obtained from Marisol (0.8%) followed by Satsuma Mino (0.8%) and Nova (1.0%). The indigenous landraces (Khoku, Banskhark Local and Sikkime) were found having same level of acidity (1.1%). The finding of low acidity in landraces are similar with reports of Paudel and Shrestha (2002); and Shrestha et al. (2012).

The blend of sweetness and sourness is measured by ratio of TSS and TA. This ratio is found highly significant among tested cultivars for both year 2016 and 2017 and for average of two years (Table 3). The highest TSS and TA ratio was recorded from cultivars Marisol (14.6) followed by Satsuma Mino (13.7) and Pongan (11.9), whereas the least was from cultivars Fortune (5.2) followed by Kara (6.7) and Page (8.3). The indigenous cultivars to hills were having more or less similar level for this ratio (10.5-11.0).

BrimA is new index of harvest maturity derived from TSS and TA. This index was also found highly significant among tested cultivars for both years 2016 and 2017 as well as mean of the two years (Table 3). The cultivar having the highest BrimA value was Pongan (8.1) followed by Marisol and Sikkime Local (7.4). Other indigenous cultivars (Banskhark and Khoku) were also at par with Pongan for this trait. The least BrimA value was obtained from cultivar Fortune (2.8) followed by Okitsuwase (4.0) and Kara (4.3).

Yield Parameters

The number of fruits harvested in the year 2016 and 2017 as well as average of two years were highly significant among the tested cultivars (Table 4). The cultivar producing fewer number of fruits was Kara (32) followed by Oraval (58) and Nova (63), whereas the maximum fruit bearing cultivar was Commune (451) followed by Okitsuwase (297) and Sikkime Local (271).

The yield/tree was significantly different among the tested cultivars in the year 2016, 2017 as well as average of the two years (Table 4). The higher yielder trees belonged to cultivar Commune (36.1) followed by Oraval (32.2) and Satsuma Mino (32.1), whereas the lower fruit producer was Page (4.1) followed by Kara (4.7) and Nova (6.7).

The productivity was also significantly different among the tested cultivars in the year 2016, 2017 as well as the average of the two years (Table 4). The highly productive cultivar was Commune (44.11) followed by Oraval (35.72) and Satsuma Mino (35.6), whereas the lowest productive cultivar were Page (4.51) followed by Kara (5.21) and Nova (7.47). The indigenous cultivars to hills (Banskarka, Sikkime and Khoku) were in intermediate level of productivity.

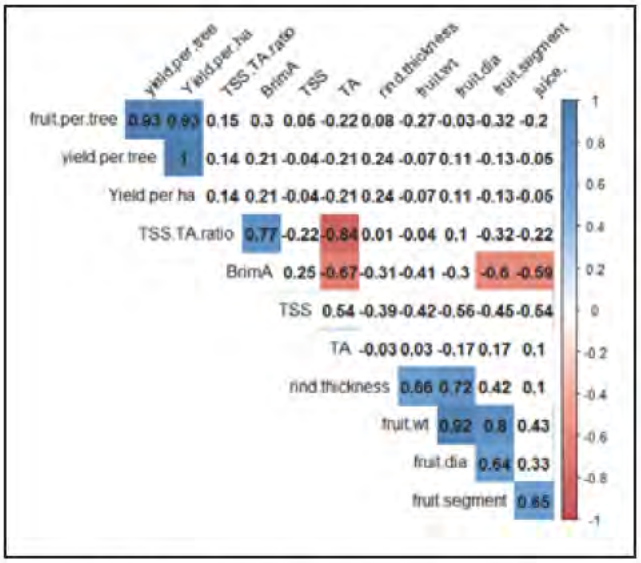

Figure 1 Correlogram of yield and quality parameters; number in graph indicates correlation coefficient and value in color indicates significant ($p > 0.01$) correlation coefficient

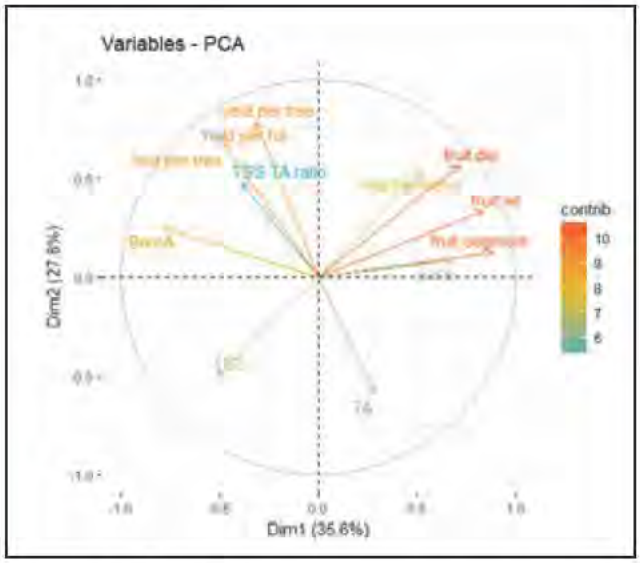

Figure 2 PCA plot of fruit and quality parameters based on pooled mean

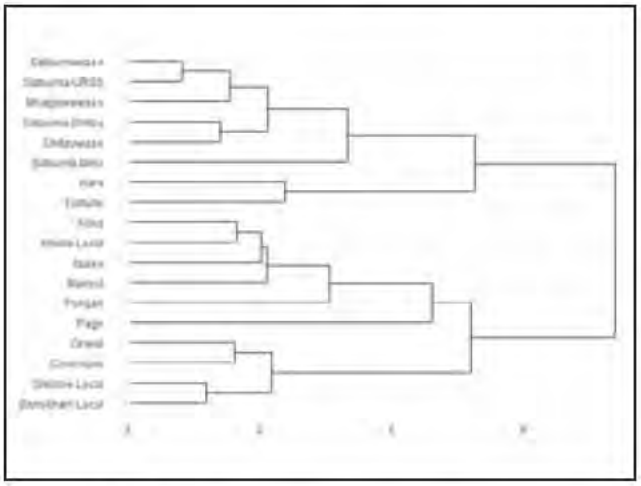

Figure 3 Dendrogram of 18 mandarin accessions based on pooled mean of two years yield and quality parameters

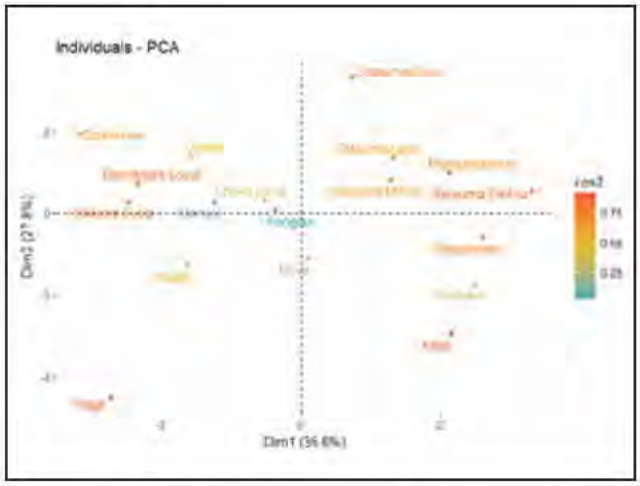

Figure 4 PCA plot of cultivars based on pooled mean of yield and quality parameters

Correlation and Cluster Analysis

The fruit quality parameters were found highly correlated ($P > 0.01$) with each other as shown by the Correlogram (Fig 1). The TSS and TA percentage were not found associated with each other as the attributes were in different coordinates of PCA plot (Fig 2) and with low correlation coefficient (Fig 1). Yield attributing parameters were also found highly correlated as shown by same graph. This is also supported by the PCA plot (Fig 2). Similarly, the fruit physical parameters (fruit diameter, weight, rind thickness and segments) were having significantly positive association with each-others as shown by > 0.6 correlation coefficient (Fig. 1) and within same coordinate of PCA plot (Fig. 2)

Two ways of classification approaches (dendrogram and PCA) for accessions were tried deploying average of two years yield and quality parameters. Most of the Satsuma or Unshiu cultivars were in similar groups as shown in dendrogram and PCA plots (Fig 3 and 4). These all cultivars were early maturing accessions with exception of

Satsuma URSS (Table 5). The hybrids accessions introduced from France into Nepal (eg Nules, Page and Nova) were just opposite of Satsuma groups in PCA plot coordinates (Fig 4) and also within same branch of dendrogram (Fig 3). Similar type of finding was also reported on these hybrid groups by Neves et al. (2018). All these cultivars were mid maturing cultivars (Table 5). The Nepali local landraces (Banskhark, Khoku) along with Sikkime, Commune and Oraval were in same group shown as in Fig 3 and 4. These were mid to late maturing ones based on performance of all 18 accessions. No accessions were observed maturing during February or March month in the hills. Hence, there is need for introduction and hybridization in search for such cultivars in Nepali context. Further some accessions like Page has shown strong biennial behavior for fruit production (Table 4) and care should be taken while promoting such cultivars.

Table 5. Maturity time of 18 accessions of mandarin tested at NCRP farm

Accession Name	Maturity time
Satsuma Okitsu	September 4th week
Okitsuwase	October 2nd week
Miyagawawase	October 2nd week
Satsuma Mino	October 2nd week
Satsumawase	October 4th week
Marisol	October 4th week
Oraval	November 2nd week
Commune	November 2nd week
Satsuma URSS	November 2nd week
Page	November 3rd week
Nules	November 3rd week
Nova	November 3rd week
Banskhark Local	December 2nd week
Sikkime Local	December 2nd week
Pongan	November 4th week
Fortune	December 3rd week
Kara	December 3rd week
Khoku local	December 4th week

Conclusion

Based on the two years data after 13 and 14 years of planting, the cultivars Unshiu and Satsuma mandarins (Okitsuwase and Miyagawawase) were found as early season harvesting (first week of October) in NCRP farm at an altitude of 1200 m height. Most of the hybrid accessions and landraces (Banskhark and Khoku Local) have shown potential to be mid-season (1st week of November) harvesting cultivars. However, all the collected accessions are

not good enough to be promoted as late season cultivars. Therefore, research system need to search for some exotic materials to be promoted as late season. Murcott and Nagpuri mandarin could be among few options to be explored in future. Further, a hybrid cultivar development system including landraces and exotic materials could be another avenue to explore in the days to come.

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Table 1. Fruit yield and quality parameter of 18 mandarin accessions tested at NCRP Dhankuta in year 2016 and 2017

Accession Name	Fruit Wt*1(gm)	Fruit Wt*2 (gm)	Av. Fruit Wt (gm)	Fruit Dia 1 (mm)	Fruit Dia 2 (mm)	Av. fruit dia (mm)	Rind thick 1 (mm)	Rind thick 2 (mm)	Av. rind thick (mm)
Banskhark Local	109.43	92.81	101.12	57.67	58.64	58.17	2.10	2.23	2.17
Commune	82.47	76.40	79.43	53.60	59.17	56.37	2.20	2.34	2.27
Fortune	135.90	127.30	131.60	59.43	65.87	62.63	3.57	1.93	2.73
Kara	118.20	175.86	132.57	57.30	73.27	62.00	1.67	2.71	2.00
Khoku Local	113.67	121.18	117.43	55.40	66.33	60.87	2.53	2.29	2.40
Marisol	134.77	92.87	113.83	63.60	62.81	63.23	2.47	2.03	2.23
Miyagawawase	131.97	117.74	124.87	60.53	65.84	63.20	2.83	2.89	2.87
Nova	119.00	110.36	114.67	58.23	62.57	60.40	1.97	2.21	2.07
Nules	87.17	71.11	79.17	53.97	54.14	54.07	2.67	1.92	2.30
Okitsuwase	132.27	128.56	130.40	60.27	68.63	64.43	2.30	2.62	2.47
Oraval	124.80	94.1	109.43	60.57	59.92	60.23	2.00	3.03	2.53
Page	63.23	-	63.23	41.10	-	41.10	1.43	-	1.43
Pongan	142.97	135.77	139.37	66.57	65.44	66.00	2.93	2.51	2.73
Satsuma Mino	171.10	114.46	142.77	65.30	65.94	65.63	2.97	2.26	2.60
Satsuma Okitsu	169.40	136	152.67	72.07	68.95	70.53	2.93	2.14	2.53
Satsuma URSS	118.67	126.49	122.60	57.90	72.59	65.23	2.07	2.56	2.30
Satsumawase	135.97	120.93	128.47	60.17	69.91	65.07	2.23	2.38	2.30
Sikkime Local	95.27	92.97	94.13	54.77	58.91	56.80	1.73	1.68	1.73
Mean	121.45	112.58	115.43	58.80	64.64	60.89	2.37	2.33	2.31
P value	*	Ns	***	**	*	***	***	**	***
LSD	52.65	-	35.18	10.13	9.91	7.39	0.58	0.57	0.37
CV%	26.15	26.19	18.39	10.39	9.24	7.32	14.81	14.66	9.69

*1 = year 2016 and 2 = year 2017

Table 2. Fruit yield and quality parameter of 18 mandarin accessions tested at NCRP Dhankuta in year 2016 and 2017

Accession Name	Seg/fruit1	Seg/fruit 2	Av. fruit segment	Juice%1	Juice%2	Av. juice%	TSS1	TSS2	Av. TSS
Banskhark Local	9.80	9.20	9.50	48.33	28.02	38.17	12.00	11.72	11.86
Commune	8.80	9.53	9.17	44.63	38.14	41.37	11.87	11.60	11.73
Fortune	12.07	9.73	10.90	50.57	45.07	47.83	13.07	12.87	12.97
Kara	10.40	12.00	11.07	46.67	51.00	48.97	11.77	13.05	12.27
Khoku Local	9.73	10.47	10.10	42.77	47.33	45.07	12.57	10.85	11.71
Marisol	8.47	8.53	8.50	38.00	50.84	44.40	10.83	10.27	10.55
Miyagawawase	11.00	11.00	11.00	52.57	46.68	49.63	7.50	10.35	8.93
Nova	11.00	10.10	10.53	48.83	45.61	47.23	11.33	11.07	11.20
Nules	8.67	9.20	8.93	42.57	41.73	42.13	10.53	11.20	10.87
Okitsuwase	11.20	11.20	11.20	50.20	45.92	48.07	8.27	10.13	9.20
Oraval	8.73	9.13	8.93	46.43	36.98	41.70	11.17	12.10	11.63
Page	9.20	-	9.20	44.03	-	44.03	12.93	-	12.93
Pongan	10.13	10.60	10.37	29.57	35.33	32.43	12.03	12.50	12.27
Satsuma Mino	11.53	11.27	11.40	53.97	52.29	53.10	9.90	10.78	10.34
Satsuma Okitsu	11.53	11.73	11.63	55.20	48.67	51.93	8.13	10.80	9.47
Satsuma URSS	10.87	11.10	10.97	50.93	47.60	49.27	9.30	9.87	9.58
Satsumawase	11.27	10.93 .929.60	11.10	51.63	45.78	48.70	9.40	9.63	9.52
Sikkime Local	9.40	9.60	9.50	48.23	39.16	43.70	11.73	11.73	11.73
Mean	10.21	10.28	10.22	46.95	43.74	45.43	10.8	11.17	11.04
P value	***	**	***	***	**	***	***	***	***
LSD	0.98	0.63	0.57	4.81	10.61	5.28	0.96	1.08	0.76
CV%	5.77	3.68	3.34	6.18	14.59	7.01	5.35	5.80	4.13

Table 3. Fruit quality parameter of 18 mandarin accessions tested at NCRP Dhankuta in year 2016 and 2017

Accession Name	TA1	TA2	Av. TA	TSS/TA ratio 1	TSS/TA ratio 2	Av. TSS/TA ratio	BrimA 1	BrimA 2	Av. BrimA
Banskhark Local	1.11	1.13	1.12	10.83	10.42	10.63	7.56	7.18	7.37
Commune	1.24	1.13	1.19	9.63	10.22	9.93	6.92	7.06	6.99
Fortune	2.92	2.17	2.55	4.50	5.95	5.21	1.37	4.17	2.77
Kara	2.22	1.36	1.99	5.43	9.65	6.71	2.89	7.61	4.31
Khoku Local	1.23	1.04	1.13	10.50	10.47	10.48	7.65	6.71	7.18
Marisol	1.01	0.56	0.78	10.80	18.29	14.55	6.81	8.01	7.41
Miyagawawase	1.35	0.77	1.06	5.57	13.39	9.48	2.09	7.26	4.68
Nova	1.06	0.99	1.02	10.80	11.97	11.38	7.11	7.12	7.11
Nules	1.06	1.06	1.06	10.17	10.59	10.37	6.30	6.96	6.63
Okitsuwase	1.90	0.69	1.30	4.47	14.63	9.54	0.66	7.36	4.02
Oraval	1.24	1.40	1.32	9.03	8.71	8.87	6.20	6.49	6.35
Page	1.57	-	1.57	8.23	-	8.26	6.67	-	6.67
Pongan	1.13	0.98	1.05	10.67	13.06	11.87	7.52	8.61	8.06
Satsuma Mino	1.02	0.61	0.82	9.73	17.68	13.71	5.83	8.33	7.08
Satsuma Okitsu	1.10	1.22	1.13	2.03	8.93	8.26	3.78	5.93	4.85
Satsuma URSS	0.98	1.06	1.02	9.63	9.27	9.45	5.40	5.60	5.50
Satsumawase	1.16	1.06	1.11	8.10	9.17	8.63	4.74	5.4	5.07
Sikkime Local	1.17	1.01	1.09	10.03	11.72	10.89	7.04	7.71	7.38
Mean	1.36	1.07	1.24	8.34	11.45	9.90	5.36	6.90	6.08
P value	***	***	***	***	***	***	***	***	***
LSD	0.27	0.21	0.25	1.69	2.40	1.65	1.45	1.24	1.18
CV%	11.87	11.69	11.95	12.21	12.61	10.05	16.25	10.83	11.70

Table 4. Fruit yield parameter of 18 mandarin accessions tested at NCRP Dhankuta in year 2016 and 2017

Accession Name	#fruit/tree1	#fruit/tree 2	Av.fruit #/ tree	Yield/tr (Kg)1	Yield/tr (kg)2	Av. yield/ tree (kg)	Yield ton/ha (1)	Yield ton/ha (2)	Av.Yield ton/ ha
Banskhark Local	236.33	291.7	264.33	26.16	26.38	26.27	29.06	29.31	29.18
Commune	416.67	485.7	451.33	34.26	37.94	36.10	38.07	42.15	40.11
Fortune	184.67	101.3	143.33	23.77	11.53	17.65	26.41	12.81	19.61
Kara	34.33	38.00	32.33	5.00	5.91	4.69	5.55	6.57	5.21
Khoku Local	224.00	115.7	170.33	25.62	12.52	19.07	28.47	13.91	21.19
Marisol	20.67	223.0	122.00	2.58	18.91	10.74	2.86	21.01	11.93
Miyagawawase	138.33	127.0	133.00	19.18	15.71	17.44	21.31	17.45	19.38
Nova	97.00	28.3	63.00	11.48	1.97	6.73	12.75	2.19	7.47
Nules	119.33	132.0	126.00	9.73	9.46	9.60	10.81	10.50	10.66
Okitsuwase	83.00	56.7	70.00	9.39	6.25	7.82	10.44	6.94	8.69
Oraval	239.33	354.0	296.67	29.41	34.90	32.16	32.67	38.78	35.72
Page	57.67	-	57.67	4.06	16.45	4.06	4.51	-	4.51
Pongan	25.67	163.7	95.00	3.83	-	10.14	4.26	18.28	11.27
Satsuma Mino	183.67	305.3	245.00	30.13	33.96	32.05	33.48	37.73	35.60
Satsuma Okitsu	146.67	147.0	147.00	2.92	19.02	10.97	3.24	21.13	12.18
Satsuma URSS	173.33	142.7	158.33	20.86	17.61	19.24	23.18	19.56	21.37
Satsumawase	224.00	185.0	204.67	29.98	20.93	25.45	33.31	23.25	28.28
Sikkime Local	205.33	336.3	271.33	19.49	29.54	24.51	21.65	32.82	27.24
Mean	156.10	193.24	169.52	17.10	19.02	17.48	19.00	21.13	19.42
P value	***	***	***	***	**	***	***	**	***
LSD	106.90	115.33	93.98	12.37	17.61	11.62	13.74	19.57	12.91
CV%	46.32	48.34	33.45	43.63	55.68	40.10	43.63	55.68	40.09