SELECTION OF ELITE MANDARIN (*Citrus reticulata* Blanco) MOTHER PLANT FROM LOCAL GENOTYPES

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ABSTRACT

A total of 26 mandarin seedling trees of 25 years age, collected from Khoku and maintained at National Citrus Research Program, Dhankuta were evaluated for four consecutive years from 2001 to 2004 for yield and fruit quality. High level of diversity was noted on marketable yield (25-131 kg), yield efficiency (0.22-1.10 kg/m^3), fruit size (66.5 – 87.8 gram), seed number (8.6-16.6) and acid content (0.81-1.40 percent) while variation on pulp percent, juice content and TSS was less. Each tree was scored in 1-5 scale for its performance on TSS, total acid, TSS acid ratio, pulp percent, juice content, seeds/fruit, percentage of big and medium sized fruits and yield efficiency. Tree number: J-90 which obtained highest score (18) was selected as mother plant. Disease indexing in Nepal and Corsica, France showed that J-90 was free from graft-transmissible diseases. The selected plant was multiplied by grafting on Poncirus trifoliata rootstocks and maintained as primary foundation mother plant under screen house at National Citrus Research Program, Dhankuta and as secondary mother plants in private nurseries in Kavre and Lamjung. Demand of disease-free saplings of this genotype is very high and is being sold by private nurseries in a price of Rs 50-125 per sapling. The yield of three years old plant has been found up to 30 kg at farmers' field.

INTRODUCTION

Citrus are the historic fruit crops of Nepal. One of the most important indicators of citrus antiquity in Nepal is the diversity in native forms. Among the citrus fruit crops mandarin (*Citrus reticulata*), sweet orange (*Citrus sinensis*) and acid lime (*Citrus aurantifolia*) are the major commercially grown in Nepal.

Mandarin is the most important citrus fruit crop of Nepal. It is grown in more than 50 hill districts of the country. Mandarin contributes about 60 percent of the total area and production of citrus fruit crops. Government statistics shows that at present area under mandarin cultivation is estimated to be 22060 hectare, production 174868 Mt. and productivity 12.0 mt/ha (MoAC, 2010. In the last 20 years area under CITRUS cultivation has increased by 178 percent where as productivity has not increased remarkably and has remained 10.2 to 11.3 tons/ha level. This productivity is relatively low compared to many other citrus growing countries (FAO, 2010). Some efforts were made in the past for *in-situ* and *ex-situ* evaluation of local mandarin germplasm (NCRP, 2004 and HDP, 1997). However, the past works could not produce conclusive results in terms of variety registration or elite mother plant selection for commercial use. As a result, use of unselected mother plants for propagation of mandarin is still a common practice in Nepal. Indiscriminate use of such unselected genotypes is one of the major reasons for low productivity of mandarin in Nepal (Roastacher, 1996).

Conventional and modern techniques have become an integral part of variety improvement of citrus worldwide. The conventional technique includes selection of elite trees from seedling originated population. It is possible in countries where high level of diversity on seedling trees exists. This can be a starting point of variety improvement from local gene pool. Other techniques of citrus improvement include hybridization and selection of selection of trees from F1 population, isolation of spontaneous mutant like limb sports (Nishiura, 1964), somatic hybridization (Saito, et. al, 1994, Grosser et al, 2008) and irradiation to reduce seediness (UCR, 2011). Khoku village of Dhankuta district of eastern Nepal is known for high quality mandarin production. Therefore, this study was undertaken to select elite mandarin mother plant from seedling trees of Khoku land races for further clonal propagation.

MATERIALS AND METHODS

Plant materials: About 25 years old seedling mandarin trees maintained at Jyamire block of National Citrus Research Program, Paripatle Dhankuta (1350 m altitude) were evaluated for their horticultural characters. From among the 105 trees maintained in the block 26 were selected for evaluation based on visual observation of vigor and production performance. These trees were raised from the seeds of a selected elite mandarin tree collected from farmer's orchard of Khoku V.D.C, Dhankuta. The trees were maintained with normal cultural practices since establishment. The trees were evaluated for four harvesting years from 2000 to 2004.

Fruit yield: Canopy volume of the tree was estimated using the formula as described by Holtzhausen et al. (1988): $V = r^2 (\pi h\text{-}1.046r)$ where, V is canopy volume of the tree, r is radius of tree canopy (half of average canopy diameter) and h is height of the tree. Yield efficiency (Kg/m^3) was calculated by dividing the marketable yield (kg) by the volume of the tree. Fruits were harvested in second week of Poush when there was complete yellow color development in all fruits. Harvested fruits were divided into four categories based on size: large (≥100 gm), medium (70-99 gm), small (50-69 gm) and unmarketable (< 50 gm). Highly malformed fruits were also considered unmarketable. Large, medium and small fruits were considered marketable ones. Total marketable yield (kg) was divided by number of fruits to determine fruit size.

Fruit quality: Nine randomly selected fruits (three from each of large, medium and small category) were used for determining total soluble solids (TSS), total acid (TA), juice content, pulp percent and number seeds per fruit. Pulp and juice percent was calculated on the basis of total weight of the fruit. TSS was determined with the help of hand refractometer whereas total titratable acidity was determined by titrating the juice with 0.01 N sodium hydroxide to the phenolphthalein end point. The total acid percent was calculated using the formula (Ranganna, 1995)

$$\text{TA \%} = \frac{\textit{Volume of titrant x Normality of titrant x Eq.wt.of citric acid x 100}}{\textit{Volume of sample x 1000}}$$

Eight traits related to yield and quality of fruits and are important on horticulture point of view were used for superior mother plant selection (Paudyal, 2003). In order to select superior tree (s) each accession was scored for these traits as given below:

TSS (^{0}Brix): >15 = 5; 13-15 = 4; 11-13.0 = 3; <11 = 2
TA (%): <0.6 = 5; 0.6-0.79 = 4; 0.8-0.1.0 = 3; >1.0 = 2
TSS/TA: >15 = 5; 12-15 = 4; 10-11.12 = 3; <10 = 2
Pulp percent: >70 = 3; 60-70 = 2; <60 = 1
Juice percent in pulp: >50 = 3; 40-50 = 2; <40 = 1
Number of seeds/fruit: < 6 = 3; 6.1-12 = 2; >12 = 1
Percent of big and medium fruits: >80 = 4; 70-80 = 3; 60 -70 = 2; <60 = 1
Yield efficiency: >1.0 = 4; 0.8-1.0 = 3; 0.6 – 0.8 = 2; < 0.6 = 1

The score of individual trait obtained by each accession was summed up and accession obtaining highest score was selected as mother plant. The selected plant was tested for graft-transmissible diseases in Corsica France, saplings were produced on trifoliate rootstocks and maintained under insect proof screen houses as primary foundation mother stock.

RESULTS AND DISCUSSION

Yield

Marketable fruit yield of 26 trees evaluated in the study is presented in table 1. The marketable yield ranged from 25.3 kg to 131.5 kg per tree. Tree No. J-82 was the highest yielder (131.5 kg) followed by J-90 (126.5 kg). Wide variation among trees in fruit productivity was reflected by high CV (36.7%) for fruit yield. Overall 85.4 percent of the total fruits produced in 26 trees were good for market. Highest percentage of marketable yield was produced in J-72 (95.4%) and lowest was in J-41 (70.7%). Proportion of large, medium and small fruits produced in different trees varied greatly (CV = 51.3%). On an average 18.2% of the fruits in sampled 26 trees were large sized, 33.0% medium and 34.2% were small sized fruits. J-90, the second highest yielder tree, produced highest proportion (41.3%) of large sized fruits

Table 1. Fruit yield of different mandarin accessions (4 years mean)

Tree No.	Marketable Fruit Yield (Kg)	Marketable Fruit yield based on size (%)			Marketable yield %	Tree volume m^3	Yield efficiency (kg/m^3)
		Large	Medium	Small			
J4	54.2	20.8	43.9	29.5	94.2	107.5	0.50
J9	78.7	32.1	44.1	19.1	95.3	111.2	0.71
J13	79.1	22.1	44.9	25.8	92.8	102.4	0.77
J16	68.2	14.5	41.3	32.8	88.6	107.4	0.63
J21	58.4	20.8	26.4	46.4	93.6	99.0	0.59
J23	65.8	14.2	23.2	51.2	88.6	135.9	0.48
J24	65.6	30.1	39.5	18.2	87.8	191.7	0.34
J26	56.0	26.0	38.1	25.8	89.9	116.4	0.48
J34	59.0	2.6	15.0	58.5	76.1	92.4	0.64
J35	95.1	8.2	18.1	48.6	74.9	169.1	0.56
J40	98.0	26.7	43.1	19.8	89.6	142.6	0.69
J41	61.1	4.6	15.7	50.4	70.7	95.7	0.64
J42	25.3	12.3	30.5	44.9	87.7	112.8	0.22
J48	69.4	20.9	32.8	34.8	88.5	88.7	0.78
J50	26.1	4.0	31.1	46.2	81.3	48.0	0.54
J52	78.4	30.0	37.4	26.1	93.5	107.3	0.73
J58	108.9	22.6	41.4	27.6	91.6	182.7	0.60
J68	59.5	19.9	22.8	35.5	78.2	101.3	0.59
J70	125.6	10.5	44.1	38.7	93.3	216.7	0.58
J71	70.5	10.2	25.8	38.4	74.4	196.1	0.36
J72	47.1	17.9	40.4	37.1	95.4	83.5	0.56
J75	111.3	11.7	27.9	35.1	74.7	178.5	0.62
J82	131.5	16.3	41.1	19.1	76.5	172.1	0.76
J84	84.5	14.4	26.8	38.2	79.4	127.1	0.66
J90	126.5	41.3	30.7	16.5	88.5	122.7	1.03
J91	96.3	19.4	32.5	23.2	75.1	145.1	0.66
Mean	76.9	18.2	33.0	34.1	85.4	129.9	0.61
Max .	131.5	41.3	44.9	58.5	95.4	216.7	1.03
Min .	25.3	2.6	15.0	16.5	70.7	48.0	0.22
CV%	36.73	51.3	28.3	34.4	9.4	31.8	26.07

Tree No J-34 produced only 2.6 % of large sized fruits. Percentage of medium sized fruits ranged from 15.0 to 44.9 percent and small sized fruits from 16.0 to 58.5 percent among the trees. Fruit productivity per unit volume of tree which is also termed as yield efficiency was highly variable (CV % = 26%) among trees.

Fruit Quality

Table 2 presents variation on fruit quality related traits of the mandarin trees evaluated in this study. Coefficient of variation (CV), which estimates the amount of variation in a sample population, was found to be varied greatly among traits. CV was very low in pulp percent (2.55%) and low in juice percent (5.13%) and TSS (5.33%). It suggests that diversity in these characters was low in the sampled population and there is less scope of tree selection on the basis of these traits. The CV on average fruit weight (7.84%) was medium while CV was found high on seeds per fruit (19.6%), total acids (16.6%) and TSS: acid ratio (15.6%).

Fruit weight ranged from 66.5 to 87.5 gram . Tree number J-71 had smallest fruits while J-35 produced biggest fruits. Pulp percent ranged from 64.3 (J-40) to 69.8 (J-16). Among the evaluated trees , J-48 was most juicy (46.5%) and fruits of J-42 had less juice (37.7%). Number seeds per fruit varied from 8.6 (J-42) to 16.6 (J-9). TSS ranged from 10.7 to 12.9 with 5.33 % CV. Variation on TA was high ranging from 0.81 to 1.4 (CV = 16.57%). Like wise minimum TSS/TA ratio (7.86) was recorded in the fruits of J-42 and maximum (14.52) was in J-21. It gives the indication that assessment of TSS value alone does not give

adequate information on sweetness of mandarin fruits rather TSS/TA ratio will be more accurate quantitative measurement of sweetness in mandarin fruit

Table 2. Fruit characteristics of different accessions of mandarin (4 years mean)

Tree No	Fruit wt. (gm)	Pulp %	Juice %	Seed/fruit	TSS (°Brix)	TA (%)	TSS/TA
J4	74.7	65.2	41.2	11.6	12.9	1.20	10.75
J9	84.5	68.5	44.5	16.6	11.4	1.00	11.40
J13	84.7	69.7	43.8	15.4	11.7	0.87	13.45
J16	73.9	69.8	42.6	8.9	12.8	0.92	13.91
J21	83.3	65.3	39.1	14.4	12.2	0.84	14.52
J23	78.5	66.8	41.4	14.7	10.7	0.85	12.59
J24	82.9	69.3	44.5	14.8	11.0	0.92	11.96
J26	87.4	68.5	41.3	15.5	11.0	0.83	13.25
J34	79.4	65.4	38.3	8.8	11.0	0.81	13.58
J35	87.8	65.6	39.9	10.6	11.4	1.30	8.77
J40	76.0	64.3	41.1	12.8	10.9	0.84	12.98
J41	72.0	69.4	41.7	8.6	11.5	0.94	12.23
J42	72.4	65.5	37.7	8.9	11.0	1.40	7.86
J48	79.5	68.9	46.5	8.7	11.2	1.20	9.33
J50	72.4	66.3	40.0	14.3	11.5	1.23	9.35
J52	83.2	66.0	41.6	14.0	11.4	1.12	10.18
J58	73.6	66.9	41.0	12.5	11.6	1.21	9.59
J68	82.9	65.2	39.3	12.3	11.6	0.84	13.81
J70	68.8	68.3	41.7	12.0	11.1	0.94	11.81
J71	66.5	67.1	39.7	9.6	12.8	0.93	13.76
J72	73.5	66.8	41.6	12.7	12.9	1.12	11.52
J75	76.5	66.3	42.5	12.6	11.5	1.10	10.45
J82	75.2	65.3	39.9	11.6	11.5	1.14	10.09
J84	67.6	64.8	37.8	10.8	11.5	0.98	11.73
J90	82.3	65.8	41.0	12.7	11.2	0.86	13.02
J91	72.9	65.2	39.5	10.3	12.0	1.10	10.91
Mean	77.4	66.8	41.1	12.1	11.6	1.02	11.65
Max	87.8	69.8	46.5	16.6	12.9	1.40	14.52
Min	66.5	64.3	37.7	8.6	10.7	0.81	7.86
CV %	7.84	2.55	5.13	19.60	5.33	16.57	15.60

Mother Plant Selection

An attempt was made to select superior tree from the evaluated accessions based on the scoring criteria described earlier. Table 3 shows the total score of seven traits used for selection of mandarin accessions. It is apparent from the score of individual accessions that differences existed in the total score, ranging from 12-18. Accession, J-90 which was the highest scorer (18) was selected as mother plant for clonal propagation. J-90 was indexed for graft-transmissible diseases such as huanglungbing, exocortis, psorosis and tristeza virus at Citrus Research Centre Corsica, France and found negative for all these diseases. Grafted saplings on trifoliate rootstocks were produced and established inside screen-houses at National Citrus Research Program, Dhankuta, demo-farm of ECARDS, Nepal, Banepa and a private nursery in Lanjung.

Plants protected by local communities possess many valuable genes. Such materials can be utilized for development of superior cultivars. In many cases the assessment of genetic diversity and varietal selection program are often carried out separately. At the completion of genetic diversity evaluation, germplasm are either conserved in gene bank or handed over to breeder for varietal improvement. At least 10-15 years are required to recommend the variety for farmers' cultivation. In case of fruit crops with long gestation period it may take more years. In this study, to make the process short *in situ* evaluation of and superior plant selection was carried out simultaneously. As Khoku mandarin of Dhankuta is known for high quality fruits, its seedling progenies established in the research farm were evaluated and a superior mother plant was selected.

There is no universal grade and quality standards for mandarin fruit but quality standards for variety selection are sharply conditioned by climate in growing areas and consumers' acceptability (Grierson and Ting, 1978). In fruit crops, fruit quality is the more important than productivity from market point of view. But growers would like more productive varieties for higher income. Therefore, both quality and productivity parameters are considered in citrus variety selection. Percent juice, acid content, sugar and total soluble solids, fruit size and weight, rind thickness, texture, color and strength are major quality parameters considered in variety selection (Gallasch, 2000). Mandarin trees used for evaluation were not uniform in their size. Obviously total yield of the tree is highly dependent on tree volume or surface area. Therefore, rather than total fruit yield, yield efficiency was used as a measure of productivity of the trees. Two systems: yield in kilograms per unit surface area (Gallasch, 2000) or per unit canopy volume of the tree (Holtzhausen , 1988) has been used to express yield efficiency of citrus trees.

Table 3 Individual and total score of seven traits used for superior tree selection

Tree No.	Pulp %	Juice %	Seed No.	TSS	TA	TSS/TA	Fruit Size	Yield efficiency	Total Score
J4	2	2	2	3	2	3	2	1	17
J9	2	2	1	3	2	3	3	2	18
J13	2	2	1	3	3	4	2	2	19
J16	2	2	2	3	3	4	1	2	19
J21	2	1	1	3	3	4	1	1	16
J23	2	2	1	2	3	4	1	1	16
J24	2	2	1	3	3	3	2	1	17
J26	2	2	1	3	3	4	2	1	18
J34	2	1	2	3	3	4	1	2	18
J35	2	1	2	3	2	2	1	1	14
J40	2	2	1	2	3	4	2	2	18
J41	2	2	2	3	3	4	1	2	19
J42	2	1	2	3	2	2	1	1	14
J48	2	2	2	3	2	2	1	2	16
J50	2	2	1	3	2	2	1	1	14
J52	2	2	1	3	2	3	2	2	16
J58	2	2	1	3	2	2	2	2	16
J68	2	1	1	3	3	4	1	1	16
J70	2	2	2	3	3	3	1	1	17
J71	2	1	2	3	3	4	1	1	17
J72	2	2	1	3	2	3	1	1	15
J75	2	2	1	3	2	3	1	2	16
J82	2	1	2	3	2	3	1	2	16
J84	2	1	2	3	3	3	1	2	17
J90	2	2	2	3	3	4	3	3	22
J91	2	1	2	3	2	3	1	1	16

CONCLUSION

Mandarin is the major fruit crop of Nepal especially grown in mid-hills. Despite economic importance and very long history of its cultivation no variety has been registered or released yet. Due to this reason saplings/seedlings are being produced from unselected mother plants both in public and private sector. To fulfill the varietal need for quality mother plants production 26 seedling trees of Khoku mandarin were evaluated for their fruit quality and productivity. Best performing tree (J-90) was selected based on pulp

percent, juice percent, seed number, TSS, TA, TSS:TA ratio, fruit size and yield efficiency. Since laboratory test revealed that J-90 was free from major graft-transmissible diseases, grafted saplings produced and established in primary mother stock blocks. The mother plants of this variety are being used for sapling production by National Citrus Research Program Dhankuta and some private nurseries. J-90 is mid-season genotype with harvesting period from Mangsir to Magh. The genotype can be registered in National Seed Board and used as commercial variety in mid-hills of Nepal. Major characteristics of J-90 are as follows:

Average fruit weight: 82.3 gram
Pulp percent in the fruit: 65.2
Juice percent in the pulp: 41.0
Number of seeds/fruit: 12.7
Total soluble solids (^{0}Brix) : 11.2
Total acid content in juice (%): 0.86
TSS/TA ratio: 13.02
Yield efficiency: 1.03 kg fruits/ m^3 canopy volume.

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