

## Studies on Grafting Methods of Low Seeded Kinnow on Rough Lemon (*Citrus jambheri* Lush.)

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**Abstract:** *Citrus* is major fruit of Pakistan. Kinnow mandarin (*Citrus reticulata*) is the leading fresh fruit cultivar grafted on soil adapted dominant rootstock rough lemon (*Citrus jambheri*). The low seeded fruits in Kinnow are on high demand in market and for juice industry. Different types of grafts were used during seedless/low seeded Kinnow germplasm propagation. This paper reports survival of various graft types in field conditions. The upper plant branches had best response in grafts. The maximum survival (92%) in field was of 'T' or side grafts followed by sprout grafts (61.4%). Leaf retention character in both scion and rootstock is important for success of sprout graft survival. Top graft survival in field was 46.1%. The embryonic plants are sensitive to harsh environments in field and only 13.8% survived which had field heat and cold tolerance. Budding was least successful in low seeded Kinnow and only 4.6% buds sprouted in field conditions. Healthy rough lemon plants with dense foliage, preferably with single stem are ideal for rootstock. The leaf sprouting is more after cold season in October side grafts as compared to leaves counted in April in side grafts made during 1<sup>st</sup> week of March. Grafting definitely helps embryo plants to acclimatize field environments.

**Key words:** Embryo grafts • sprout grafts • budding • bud sprouting • leaf retention

### INTRODUCTION

*Citrus* has number one position in area and production of fruits in Pakistan. It is grown on area of 192.3 thousand hectares with the production of 2458.4 thousand tones [1]. Kinnow mandarin is the leading cultivar having seed variability and seedless trait [2]. Thirty different geo-ecological rough lemon types have been reported [3]. In this work, grafting is the combination of Kinnow scion and rough lemon stock. The Kinnow and rough lemon tissues grow together upon uniting their cut parts and produce a vegetative plant of Kinnow growing on rough lemon roots. The selected rough lemon plants showed high compatibilities with Kinnow upon grafting if the stock had dense foliage preferably with single stem and low spine density. Kinnow plants used as scion have low seeded trait (0-10 seeds/ fruit) in open pollination in mixed *Citrus* plantings. Grafting of Kinnow on rough lemon is done to adjust the scion variety to the local agro-climatic conditions of Punjab, to reduce the juvenile period especially the fruiting time and to control the shape of the plant. Rough lemon is well suited rootstock for lemons and limes but not for mandarins and orange cultivars. This stock ensures proper vigor and

productivity in scion but gives very poor fruit quality [4]. It was also reported that rough lemon is suitable rootstock for Kinnow. The increase in size of scion increased the percentage of successful grafts. Shoot tips pre-treated with antioxidants and plant growth regulators increased the percentage of successful grafts compared with the control [5]. Rough lemon has rapid growth, early production and greater adaptability to poor soils [6]. Previously we Altaf and Iqbal [7] reported grafting of sprout/shoot apex/nucellar embryo of Kinnow tissue having seedless trait on rough lemon seedlings. Normal healthy and vigorous embryos with balanced germination were top grafted on 2-18 months old soil established healthy seedlings with 76% survival. Rough lemon seedlings with dense foliage, healthy, green, vigorous stem from fleshy vegetative shoots with sprouting leaves, balanced shoot-root growth were found ideal for grafting shoot apical meristem (SAM) with 6 inches top stem with leaves having axillary meristem showed best graft survival (85%) and good source of micro plant propagation [7]. In this manuscript different types of grafting methods is compared like embryos of low seeded Kinnow fruits, sprouts of low seeded Kinnow plants, budding, top grafting and stem 'T' grafts etc. All scion material was

collected from low seeded Kinnow plants, to have an idea of most suitable scion tissue and method of grafting on rough lemon.

### MATERIALS AND METHODS

**Embryo grafts:** The seeds of low seeded fruits were used to raise the seedlings aseptically in MS medium [8] supplemented with GA@1mg L<sup>-1</sup> [9]. The growing seedlings with balanced germination of shoot and root were top grafted on six months rough lemon seedlings growing in pots. The humidity was maintained by covering the plants with transparent polyethylene bags and humidity was gradually released after one month. The plants were kept initially in shade for three months. After hardening process, these were transferred to field.

**Sprout grafts:** The sprouts of low seeded Kinnow branches were top grafted on rough lemon seedlings growing in pots. The tissue joint was covered with parafilm and then whole sprout was covered with transparent polyethylene and the humidity was gradually released after one month of grafting. The sprouts grafted on rough lemon were acclimatized for another two months and then transferred to field.

**Top grafting:** The stems of rough lemon were horizontally excised at height of 9 to 12 inches from soil level and the low seeded Kinnow branch pieces containing few buds were top grafted on these stocks. The humidity was maintained by polyethylene covers which was gradually released after one month. The grafted plants were transferred to field.

**Bud grafts:** Healthy, disease free, round in shape branches with dormant buds were collected from the canopy of the low seeded Kinnow plants. The buds were excised and T-budded on the 18 months old rough lemon plants, growing in the field.

**Side grafts:** Healthy branches from the top of the low seeded Kinnow plants were collected and side grafted on the 1-3 years old rough lemon plants growing in the field.

### RESULTS AND DISCUSSIONS

All the scion material used was from low seeded Kinnow plants. The maximum graft survival (92%) in field was from 'T' or side grafts in which scion stem from upper plant branches was used. The selected branches were

Table 1: Graft type survival in field

Type of graft	Total grafts	Survival in field	Survival(%)
Embryo	819	113	13.8
Sprout	523	321	61.4
Top grafts	152	70	46.1
Budding	325	15	4.6
T-Graft	528	487	92.0

Table 2: Rough lemon stem Diameter (D) and Leaves (L) counted in end of April grown on early march T grafts

No. of grafts	*D. Range (cm)	Average *D (cm)	Leaf Range	Average Leaves
24	0.8-1.0	1.07	9-24	16.0
44	1.1-1.5	1.28	6-23	12.2
28	1.6-3.0	2.52	9-24	15.4

round and were from previous sprouting having conspicuous buds in stem. The triangular and fresh branches were avoided. Sprout grafts having leaves on stem were next to side grafts in field survival (61.4%). Here leaf retention character is very important during grafting (joint heating time). Leaf retention is a genetic trait in *Citrus* [10]. It helps in overall plant survival. There is evidence for a genetic basis of the phenomenon of leaf retention in other plants [11]. In sprout grafts, growth is usually from terminal portion of the sprouts in the field. Top grafts survival comes after 'T' grafts and sprout grafts in field conditions and it is 46.1%. Rough lemon with thicker stem is usually used in top grafting. Healthy sprouting grafts on healthy rough lemon plants ensure healthy Kinnow plants in orchard. Embryos can be successfully grafted on rough lemon nursery plants but their survival in the field is very low (13.8%) because the embryos are derived from single cells and most of the embryos derived plants did not survived in harsh environments in hot summer and severe cold of winter season. Only those embryonic plants survived which had tolerance to extreme environmental conditions. The embryonic plants undergo juvenile phase while a grafted plant using a mature scion will not have the years of juvenility that a seedling would have. The embryonic plants are less branched as compared to plants derived from side grafts. When two plants of different genotype are grafted, the degree of adaptation or aging depends on the plasticity of both partners to perform in the enforced symbiosis [12]. The lowest survival in field was only 4.6% for budding as most of the buds never sprouted although they remained green for long time. From the results given in Table 1, it is evident that 'T' or side grafts were most successful and budding was comparatively less

Table 3: October, 2007 grafted Rough lemon stem diameter (D) and sprouted leaf count in March, 2008

No. of grafts	D. Range (cm)	Average *D (cm)	Leaf Range	Average Leaves/graft
52	1.0-1.5	1.30	15-82	32.6
32	1.6-2.0	1.81	15-180	56.0
20	2.1-2.5	2.26	40-216	142.8
12	2.6-3.0	2.93	115-151	127.6



Fig. 1: Embryo grafts



Fig. 2: Sprout grafts



Fig. 3: Top grafting



Fig. 4: Budding



Fig. 5: Side grafts

successful because of its low frequency of sprouting. Diameter of rough lemon seedlings used was 0.8-3.0cm and the average number of leaves in side grafts done in March was 12.2-16.0 after one month (Table 2). Chaudhry *et al.* [13] measured the seedling height and diameter of different rootstocks and concluded that rough lemon seedlings were more vigorous than Rangpur lime and mandarin seedling. Nasir *et al.* [4] found that graftage success percentage of Kinnow scion budded on various rootstocks during mid September, 2004 and 2005 is non-significant. Rangpur lime showed maximum grafting success (95.06% during 2004 and 95.83% during 2005) followed by rough lemon (92.06% during 2004 and 93.35% during 2005). The stem diameter range between 1-3.0cms and the average leaves per graft ranged 32.6-142.8 in March and these grafts were made in October (Table 3). It is concluded that major leaf sprouting happens in March and it is increased after cold season as happened in

October grafts. The scion material was from the upper branches of the plants. Halim *et al.* [14] concluded that bursting of inserted buds was significantly slower in buds taken from (a) older branches (b) shoots produced under winter conditions and (c) basal rather than apical buds on the same shoot.

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