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TOWARDS A SEEDLESS CULTIVAR OF KINNOW MANDARIN VI. PRODUCTION OF SEEDLESS PLANTS BY SPROUT/ SHOOT APEX/EMBRYO GRAFTS

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Abstract

The work reported relates to 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 (top) 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.

Introduction

Somatic genetic changes leading to seedless trait coupled with fixation of seedlessness in plant by shoot apex/sprout/embryo grafts on well established healthy rootstock seedling is potentially valuable for creation of new cultivars with quality fruit. Somatic variability develops primarily due to accidents of cell divisions at mitosis and can produce a permanent change in the clone. Somatic variations occur randomly at very low rates and hence the probability of detection is low. Since Kinnow is dominant commercial cultivar, which involves literally billions of plants, the probability of desirable mutations to occur spontaneously somewhere in the clone is high.

Shoot apical meristem grafts are successful in *Citrus* (Altaf *et al.*, 2001) and in Kinnow mandarin (Altaf *et al.,* 2002). Shoot apex advantage is that these are from mature trees, so avoid juvenile characteristics like spine and long vegetative phase of nucellar plants. *Citrus* shoot tip micro grafts have been used for scion rootstock compatibility reaction (Edriss & Burger, 1984), for early detection of graft incompatibility relationships (Jonard, 1986), for virus elimination (Navarro *et al.*, 1975), for micropropagation (Mosella & Ascui, 1985) for induced mutations (Liu, 1983), for overcoming the problem of nursery transplant of *in vitro* regenerated plantlets (Hazarika *et al.*, 1999) etc.

The objective of the present study was fixation of seedless trait by shoot apex/sprout/nucellar embryo grafting of seedless Kinnow tissue on rootstock seedling. The natural existing Kinnow clonal mutations for seedless trait if selected carefully and grafted on established healthy rootstock seedlings is the only way to propagate desired quality fruit. Since shoot apex/sprout is not responsive to adventitious rooting and can not be readily or economically propagated by standard methods as natural rate of mutated tissue increase is relatively slow compared to normal (wild type) Kinnow mandarin and where commercial demands stand by getting a seedless fruit cultivar on the market in the shortest possible time. Grafting is also essential for early fruiting and control of production since rough lemon is native and the best soil adapted with dominant root

system in the Punjab, Pakistan. This paper reports on the experience of micrografting low seeded/seedless Kinnow mandarin shoot apex/sprout/nucellar embryo on to rough lemon which is part of efforts to produce seedless fruit clones.

Materials and Methods

The trees/branches of Kinnow orchards were screened for low seeded/seedless trait from different parts of Punjab, Pakistan. Low seeded fruit embryos were cultured according to the procedure given by Altaf *et al.*, (2002). Shoot apex/sprouts/embryos were top grafted on soil established rough lemon seedlings. After joining the cut surfaces of scion and stock by parafilm (Sigma), the whole grafted portion was covered with transparent polythene bag until healing of the cut portion. Humidity was gradually released after 15 days and grafts were properly cared during acclimatization procedure. Experiments carried out are described below:

1) Embryos were grafted on 2 – 18 months old seedlings to know the suitable age of root system for embryo grafting. The maximum successful grafts after 45 days were noted on 6-month old rootstock seedling (Table 1).

Table 1. Effect of rough lemon age on graft survival* of normal healthy embryo.

Seedling age (months)	Survival (%)
2 (leaves removed)	31 (62)
2	23 (46)
4	27 (54)
6	38 (76)
8	25 (50)
12	27 (54)
15	35 (70)
18	32 (64)

* 50 embryos grafted per treatment.

2) One year old rough lemon plants were divided into six groups, each with 50 seedlings according to their physical appearance with the objective to screen the proper rootstock morphological characteristics for grafting. The data was recorded after 50 days (Table 2).

Table 2. Effect of one year old seedling morphology on sprout graft* survival.

Rough lemon	Survival (%)
Balanced shoot-root growth	40 (80)
Tap root with few hairs	11 (22)
Stem with dense foliage	47 (94)
Seedling with broader leaves	38 (76)
Vigorous with sprouting leaves	45 (90)
Seedling with leaf abscission trait	3 (6)

* 50 grafts per treatment

3) Different embryo types from low seeded fruits were grafted on top of six months old rough lemon seedlings and embryo graft survival was noted after 50 and 100 days (Table 3).

Table 3. Effect of embryo structure on graft survival on 6 month old rough lemon.

E Morhogenesis	E grafted	50 days Survival (%)	100 days survival (%)
N with 2, 3, 4, 5 leaves	5950	5709 (96)	4662 (78)
E with double mid-rib	115	53 (46)	41 (36)
E with elongated root	463	312 (67)	217 (47)
E without root	88	73 (83)	61 (69)
Meristemless E	25	4 (16)	Nil
E with narrow leaves	54	13 (24)	6 (11)
E with 2 stem	6	5 (83)	4 (67)
E with one leaf	367	128 (35)	92 (25)
Weak embryo	55	11 (20)	3 (5)
N large E vigorous growth	73	71 (97)	68 (3)
E with notched leaves	72	24 (33)	17 (24)

E = Embryo N = Normal Embryo

4) Shoot apices in different growth phases and with variable stem sizes were top grafted on one year old rough lemon seedlings at different times of year. The data of shoot apex survival was noted after 50 days (Table 4).

Table 4. Effect of shoot apical meristem (SAM) morphology with different stem sizes on graft survival* on one year old rough lemon.

SAM	Survival (%)
With sprouting leaves	3 (15)
With dormant bud	15 (75)
With reproductive bud	13 (65)
With mature leaf	13 (65)
With 3" top stem.	11 (30)
With 6" top stem	17 (85)
With 8" top stem	16 (80)

20* SAM per treatment

5) Shoot apex was harvested throughout the year in different months and was top grafted on one year old rough lemon. The graft survival data was recorded after 50 days (Table 5).

6) Variable stems of terminal branches as soft, semi-hard, green, brown with steaks, angular, round, with or without spines, terminal and water sprouts were top grafted onto one year old rough lemon seedlings for studying the impact of vegetative characteristics of sprout stem on its successful union and growth. The data was recorded after 50 days of grafting (Table 6).

Table 5. Effect of timing of shoot apical meristem (SAM) harvest on graft* survival on one year old rough lemon.

Month	Survival (%)
January	13 (26)
February	37 (74)
March	23 (46)
April	25 (50)
May	14 (28)
June	11 (22)
July	17 (34)
August	34 (68)
September	38 (76)
October	36 (72)
November	21 (42)
December	5 (10)

50* grafts of SAM with 2 inches stem per month.

Table 6. Effect of type of sprout on graft* survival on one year old rough lemon.

Sprout	Survival (%)
Terminal soft green round stem	15 (75)
Terminal brown round stem with streaks	8 (40)
Terminal semi-hard round stem	11 (55)
Terminal angular stem	7 (35)
Terminal angular stem with spines.	4 (20)
Angular water sprout	9 (45)
Round water sprout	12 (60)

20* sprouts per treatment

Results

The data clearly indicated that embryos could be top grafted on 2 – 18 months old soil established healthy seedlings (Fig. 1). The maximum survival of normal healthy embryo graft was 76% and minimum was 46% (Table 1). If the leaves of two months old rough lemon seedlings were removed immediately after grafting the embryos, it had no negative impact on embryo union and survival (62%) on rough lemon stem. However older seedlings required leaves. Removal of all leaves reduced the union and growth of graft.

Since rootstock seedlings have variable morphology, the seedling type has influence on sprout graft survival (Table 2). The maximum grafts (94%) worked with rough lemon seedlings having dense foliage followed by healthy, green, vigorous stem with sprouting leaves. Balanced normal growth of rough lemon with healthy top-root growth and the seedlings with dark green broader leaves were also good for graft union and survival.

Poor grafting responses were observed on rough lemon seedlings which had tap root system and some how could not develop dense root system, in fact 3 – 6 poorly developed roots emerge from the main root. Grafts could not survive (6%) on rough lemon seedlings, which had leaf fall tendency. Leaf abscission has a negative impact on graft survival.

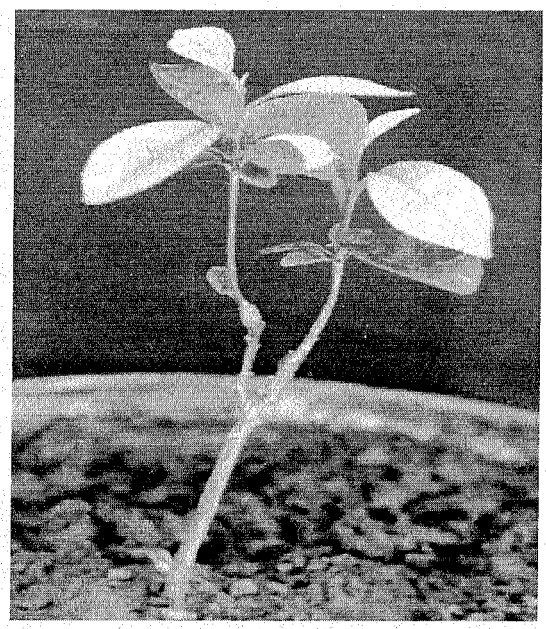

Fig. 1. Embryo graft.

Different embryonic shapes are in different frequencies in fruits collected from various orchards of Punjab. Embryo morphology had a definite effect on graft survival. The normal balanced germinated embryos have best survival upon grafting (Table 3). Abnormal embryonic growth with the exception of embryos without root development has poor grafting responses and embryo graft survival behaviour especially the meristemless embryos, which could not survive after grafting despite all efforts. Shoot apical meristem (SAM) is in different physiological and morphological conditions and the size of terminal stem with SAM has impact on survival and growth of grafts. SAM with six inches top stem have best graft survival (85%) followed by SAM with dormant vegetative bud (75%). SAM with reproductive bud and with mature leaves also survived upon grafting (Table 4). SAM with reproductive bud, if successfully grafted, it never reverted back to vegetative bud and ultimately died. The poor grafting response was with SAM sprouting leaves, which are delicate and difficult to survive under stress conditions.

Since SAM has different growth phases throughout the year, two inches SAM was harvested and top grafted on one year rough lemon seedlings. The best results were obtained in August 68% to September 76% and Feb. 74% - April 50% (Figs. 2 and 3) (Table 5). Poor responses were in December 10% which may be due to SAM being not in proper physiological state because of cold weather.

The scion stem physical characteristics definitely have influence on graft union and survival. The data indicated that sprout morphology especially the healthy green round stem has high compatibility to rough lemon (Table 6). The best sprouts were with terminal soft green round stem followed by round water sprout and terminal semi-hard round stem (Figs. 4 and 5). Terminal angular water sprouts also had graft survival to some extent but a round stem is more proliferative as compared to angular stem in Kinnow mandarin.

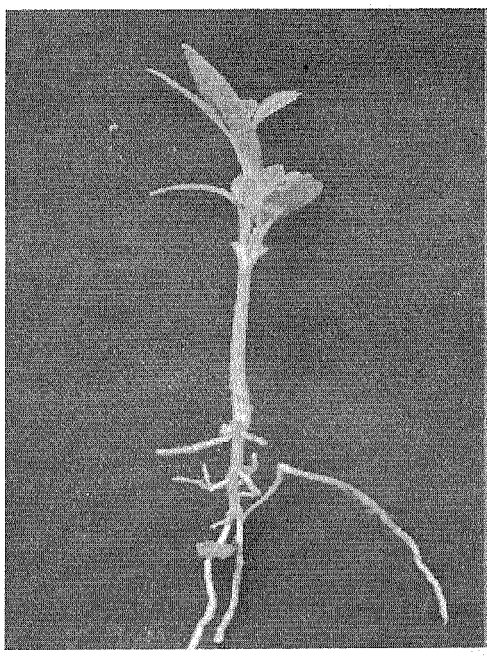

Fig. 2. Shoot apex graft.

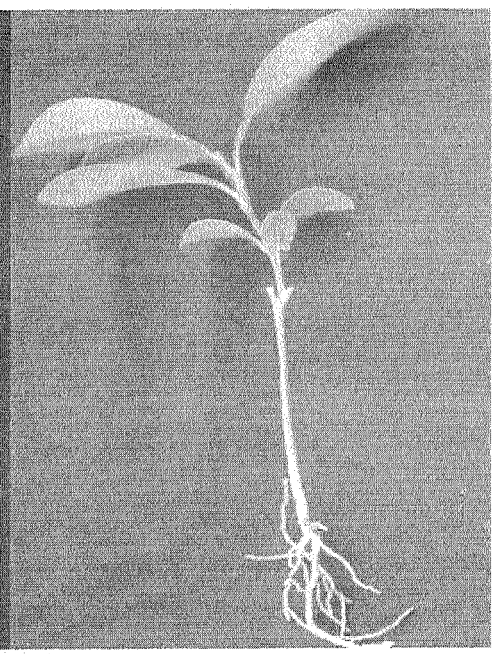

Fig. 3. Shoot apex graft

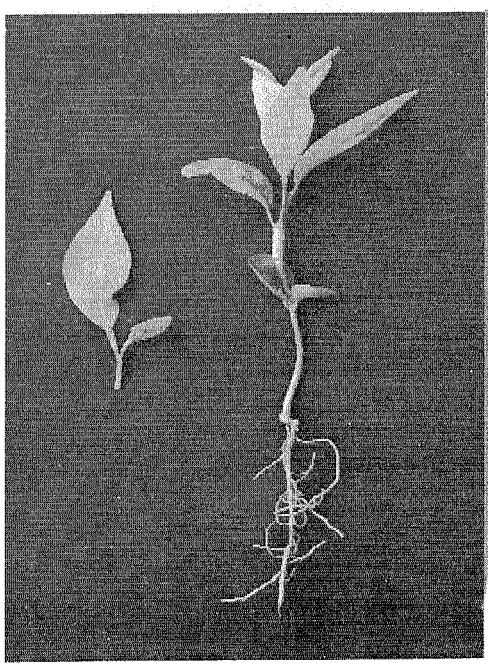

Fig. 4. Sprout graft.

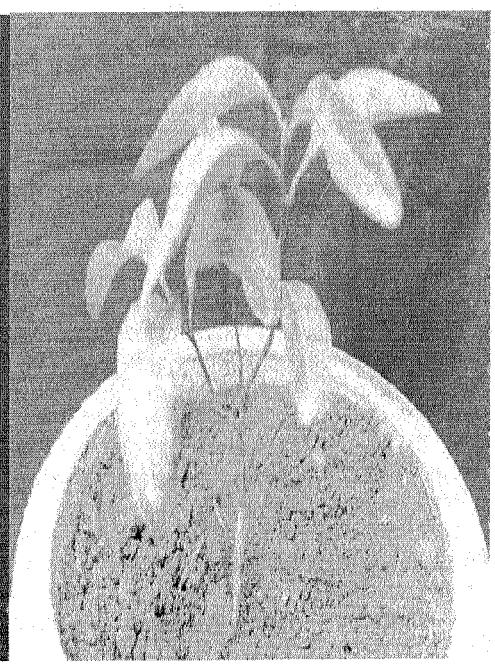

Fig. 5. Sprout graft.

Discussion

Rough lemon, *Citrus jambheri* is the native, well adapted and dominant rootstock in the Punjab, Pakistan. The species has different strains and morphological variability in seedlings. A proper rootstock seedling selection is the major consideration because it is the root system of budded tree, supporting the tree, responsible for absorption of water and nutrients, providing storage of carbohydrates produced in the leaves and synthesis of certain growth regulators, adapting the scion to particular soil conditions and potentially providing tolerance to some diseases. Grafting is an effort also to adjust the season of crop maturity and to secure earlier, more abundant, more uniform fruiting. The stock and scion must be compatible and capable of producing long lived healthy and productive trees. Straight, smooth bud unions of grafts indicate thorough congeniality or vegetative compatibility. Scion vigor, yield and juice quality generally are of greatest interest in rootstock development. Yield is the most important factor because of its strong relationship to profit. Other effects of handling heterogenic material and also in a tree with root system of one sort on top of another, the effects in relationships are hardiness, rate of growth, ultimate size, precocity, prolificness, quality and flavour of fruit and ripening season.

As rough lemon from Punjab has extreme variability, it is clear from this work that healthy, balanced top root growth of rough lemon seedlings having dense foliage, green vigorous stem with sprouting leaves and with good spread net work of root system are ideal for grafting low seeded/seedless strains of Kinnow mandarin. Kinnow also has clonal variability in all fruits characteristics including developed and undeveloped seed number. Fixation of seedless trait by shoot apical meristem with leaves having axillary buds on stem portion are best for micro plant propagation. Similarly, sprouts with healthy, green, round, terminal soft stem with healthy leaves are ideal for grafting from the twigs carrying seedless trait. Vigorous growth of normal balanced germinated embryos from low seeded fruits has maximum survival on healthy rough lemon seedlings. This work of shoot apex/sprout/embryo grafting helped in separation of different strains of Kinnow mandarin carrying low seeded/seedless trait. Probably the most important determinant of fruit quality under the control of grower is the cultivar planted. The developed plants maintain the morphological characters of the selected mother tissue but there are morphological variations in vegetative characters among the plants produced. Before commercial propagation of these clonal selections, it is better to ascertain the productivity and the qualitative characters of fruits to avoid any negative mutations that frequently occur in *Citrus*. Selection of natural mutations has been normal practice in *Citrus*. Many of these mutant types have been preserved without change as cultivars through vegetative propagation grafting (Powell, 1995) and through asexual (nucellar seed production), where the offsprings are genetically identical to the female or seed parent. Nucellar varieties have been developed from all the *Citrus* commercial cultivars and are used because of their freedom from viruses (Hartmann *et al.*, 1990).

Induction of seedlessness in Kinnow mandarin has been the major objective of this study. Gametophytic sterility, self incompatibility, and early ovule abortion are responsible for the absence or scarcity of seeds in fruit (Ueno, 1986) which may also be due to differences in genomic organizations due to inversions, translocations etc., (Hearn, 1994).

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