

STUDIES ON COMBINING ABILITY OF CITRUS HYBRIDS WITH INDIGENOUS COMMERCIAL CULTIVARS

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Abstract

An experiment was conducted in which introduced citrus hybrids (Orlando tangelo, Minneola tangelo, Saminola tangelo, Honey mandarin, Pixie mandarin and Frost dancy tangerine) were crossed with Mosambi, Kinnow and Duncan grapefruit as male parents. Pollen viability and pollen germination studies were also conducted. Pollen viability was maximum in Pixie mandarin (92.81%) while least in Frost dancy tangerine (FDT) (16.27%). Pollen tube growth studies revealed non-significant difference as pollen tube travelled 9.16, 5.80 and 4.70% of the gynoecia, in tangelos, mandarins and tangerine hybrids respectively. Kinnow generated the best value as the male parent with Honey mandarin and Orlando tangelo, while the performance with other hybrids was inconsistent as for as specific combining ability (SCA) was concerned. Although, Kinnow belongs to mandarins yet, great variability was found with Pixie mandarin for combining ability. Kinnow proved as a better pollinizer for all the experimental hybrids as for as general combining ability (GCA) is concerned. Mosambi as a male parent behaved inconsistently with experimental hybrids for fruit setting.

Introduction

The importance of citrus to agriculture and the world's economy is demonstrated by its wide distribution and large scale production. It is liked very much due to high vitamin C contents, mineral matter like calcium, dietary values and particularly therapeutic traits. At the moment, citrus is being grown in Pakistan over 185.4 thousand hectares with annual production of 1.67 Million ton (Anon., 2005). The agro-ecological conditions of Pakistan, especially prevailing in Punjab and some parts of NWFP are best suited for the production of citrus cultivars. The scope, therefore, exists for further expansion of the industry through the introduction of early or late varieties. Introduction of new varieties, with better qualitative and quantitative traits, is need of the time for success and improvement of the citrus cultivation. But, there are several problems related to the newly introduced hybrid cultivars; one of these problems is unfruitfulness leading to low yield. It will also be helpful to promote the prolonged availability of standard quality fruits to strengthen the citrus industry of Pakistan.

Introduction of Kinnow and Feutrell's Early are the glowing examples. Similarly introduction of new varieties with better qualitative characteristics are inevitable for the establishment of business and to improve the commercial cultivation of citrus fruits. Many problems are related to the newly introduced cultivars particularly hybrid cultivars; and therefore, studies about some anticipated problems of new cultivars are highly desired for the successful future of the industry.

Among other disorders, unfruitfulness is also not an uncommon feature of hybrid cultivars that may be due to several reasons; the most important is self-incompatibility (Oppenheimer, 1948; Reece & Register, 1961). Fruitfulness of a cultivar varies according

to the prevailing pollination conditions in the orchard. Unfruitfulness may also be assigned to decrease in fecundity of pollen as a consequence of sterility or inability of pollen to germinate on the stigmatic surface (Gul & Ahmad, 2006). As pollen grains of different plants require different growth media like water, sugar, inorganic salts and vitamins for successful germination (Khan & Perveen, 2006), these specific requirements may or may not be fulfilled if pollen of one variety or a hybrid is used for pollination of other cultivars. Therefore, the importance of open pollination in the production of citrus fruits depends a great deal upon the cultivars in question.

Some hybrid varieties were introduced few years back which have started blooming in experimental plots and some of them seems to be early and therefore may be recommended for commercial cultivation in the near future. These are however, unpredictable about their productivity and therefore it is feared that being hybrid they may not produce well, due to some locally uninvestigated internal problems, like self-unfruitfulness. The present endeavor was planned with an intention to find out the pollination requirements, causes of unfruitfulness if any, and to determine the extent of incompatibility in some citrus hybrids in advance, which may be helpful for future planning of citrus industry. To improve citrus cultivation and to overcome the difficulties in citrus cultivation, which may otherwise end with colossal failure, such studies are very essential to start industry on a sound footing. This problem has, therefore been taken-up towards a planned query about unfruitfulness and incompatibility of introduced citrus hybrids.

Material and Methods

The research work was carried out at the Experimental Fruit Orchard, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Three trees, 8-10 years of age were selected from each of the six newly introduced citrus hybrids, randomly. The trees were of uniform vigour and exposed to similar environmental conditions. The hybrids selected were: Orlando tangelo (Duncan grapefruit x Frost dancy tangerine), Minneola tangelo (Bowen grapefruit x Frost dancy tangerine), Saminola tangelo (Duncan grapefruit x Frost dancy tangerine), Honey mandarin (King mandarin x Willowleaf mandarin), Pixie mandarin. (F_2 -of Kincy [Kincy=King mandarin x Frost dancy tangerine]) and Frost dancy tangerine (Nucellar selection from Frost dancy tangerine).

Pollen viability test: Failure of fruit formation may be assigned to sterility or inability of pollen to germinate on stigma surface. Therefore, pollen viability test was performed, using acetocarmine as suggested by Darlington & LaCour (1962). Anthers from mature flower buds were collected and stained in 0.5% acetocarmine on a glass slide. Counts of stained and unstained pollen grains were made with the help of a tally counter.

Pollen tube growth studies: Pollen tube growth studies were conducted to observe the extent of incompatibility by employing the technique described by Martin (1958). Flowers of the hybrid varieties were artificially pollinated with known source of pollen i.e., Kinnow, Mosambi and Duncan grapefruit. At least 10 gynoecia of the pollinated flowers of each hybrid were picked after 24 and 48 hours. Then these were fixed in a fixative solution (1:8:1, formaline: alcohol: acetic acid). These were then washed in tap water and treated with 8N NaOH for 8 to 24 hours to clear and soften the tissues to permit adequate penetration of the dye. Then these were kept in distilled water for at least

one hour to remove NaOH. Staining was accomplished in 0.1% solution of aniline blue dye in a 0.1 % K_3PO_4 for 4 hours. Styles were mounted on a glass slide in a few drops of staining medium and finely crushed by a needle after placing a cover slip over the style. Styles were illuminated with UV light (356 nm) using Zeiss epifluorescence microscope (Ton & Krezdorn, 1966), which fluoresced bright yellow to yellow green for callose of the pollen tube and the background tissues in pale grey or blue colour. Pollen tube length was then measured and photograph was made using Zeiss MC 63 camera with 35 mm film.

Combining ability: Combining ability was determined by counting the percentage of fruit set as a result of pollen from different sources. General combining ability shows the overall performance of a pollen source to set fruit of any of the female cultivar in the experiment. Where as specific combining ability reveals the performance of a particular pollen source with individual female experimental hybrid. Data in this regard were subjected to statistical analysis and general combining ability (GCA) and specific combining ability (SCA) values were calculated by the method demonstrated by Simmonds (1981). Specific combining ability (SCA) was determined by keeping each pollen parent in one set, thus the total data were grouped into three sets keeping in view the behavior of a single pollinizer for different female hybrids.

Results and Discussion

Pollen viability: Pollen viability was maximum in Pixie mandarin (92.81%), statistically similar to Honey mandarin (87.56%), Minneola tangelo (86.21%) and Orlando tangelo (77.93%) (Fig. 1). Saminola tangelo and Frost dancy tangerine exhibited least pollen viability i.e., 49.29 and 16.27%, respectively. A wide range of pollen viability has already been reported in different citrus varieties (Khan, 1964; Reuther, 1967).

Pollen tube growth studies: Rate of pollen tube growth in the style indicates the gametophytic incompatibility of the cultivar in question. Hybrids were categorized into two groups based on the length of the stylar portion, tangelos with 12,000 while mandarin and tangerine hybrids with 10,000 microns.

Tube length of Mosambi pollen in the gynoecia of Pixie mandarin was 580 microns after 24 hours of pollination (Fig. 2). So in comparison to gynoecia length there was negligible extension of pollen tube in the gynoecia of the hybrid. Kinnow pollen tube traveled a length of 470 microns in Frost dancy tangerine stylar tissue (Fig. 3), while tube length of Mosambi and Duncan grapefruit pollen was far less at the same time (after 48 hours of pollination). Among the tangelo hybrids, maximum pollen tube length (1100 microns) was noticed in Orlando tangelo with Kinnow after 48 hours of pollination (Fig. 6), while in Minneola and Saminola tangelo after 24 hours of pollination with the same pollen source the tube length was recorded upto 1040 and 450 microns respectively (Fig. 4 & 5). Measurements after 48 hours of pollination revealed that the three pollen sources behaved almost in the same manner with all of the hybrid cultivars.

So it can be concluded that there was no difference in pollen tube elongation as tube traveled only 1100, 580 and 470 microns, which amounted to 9.16, 5.80 and 4.70% of the gynoecia, in tangelos, mandarins and tangerine hybrids respectively, with all the pollen sources.

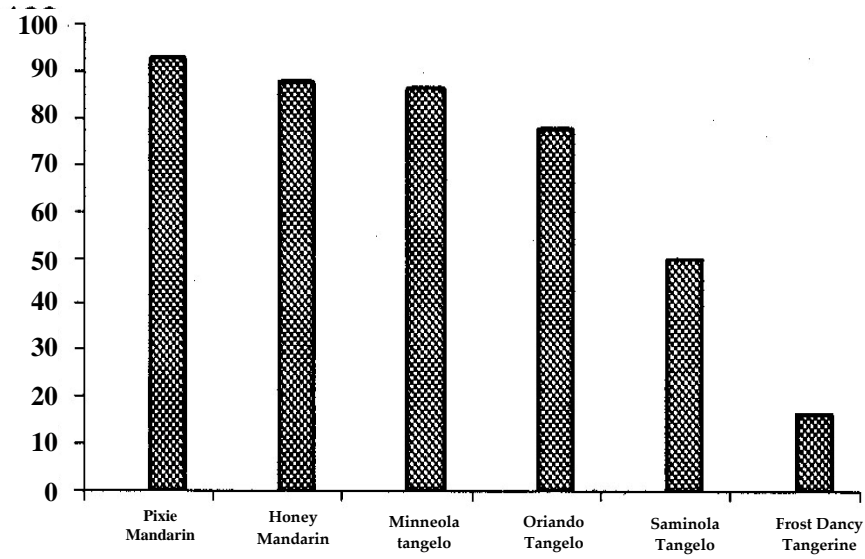


Fig. 1. Pollen Viability of Citrus Hybrids.

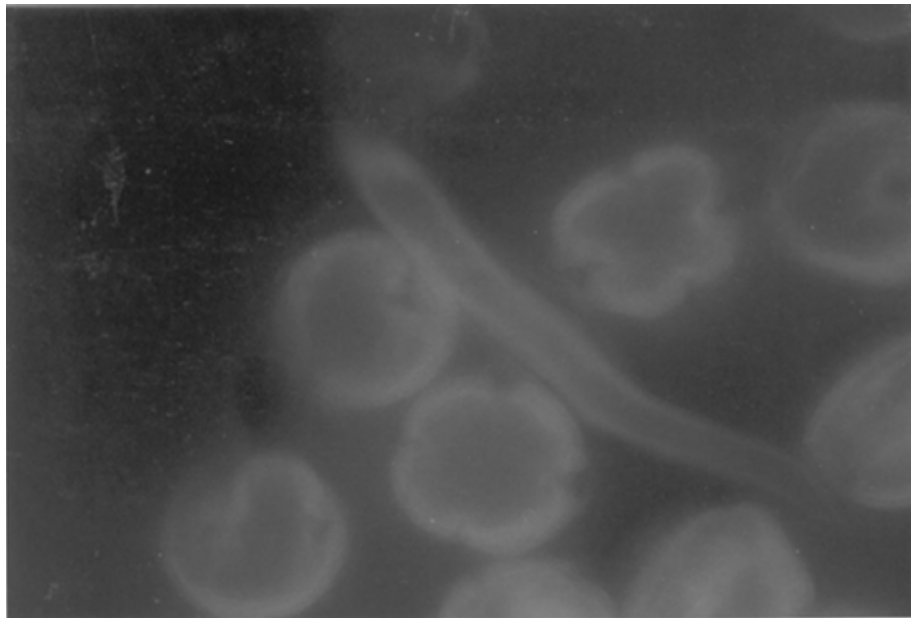


Fig. 2. Tube growth pattern of Mosambi pollen in Pixie style 24 hours after pollination (580 microns).

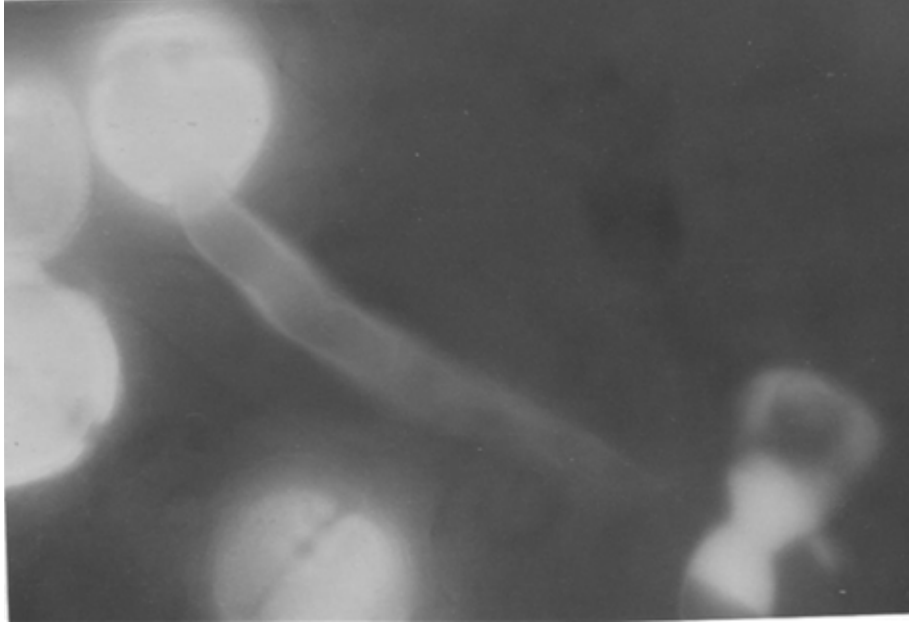


Fig. 3. Tube growth pattern of Kinnow pollen in the style of FDT 48 hours after pollination (470 microns).

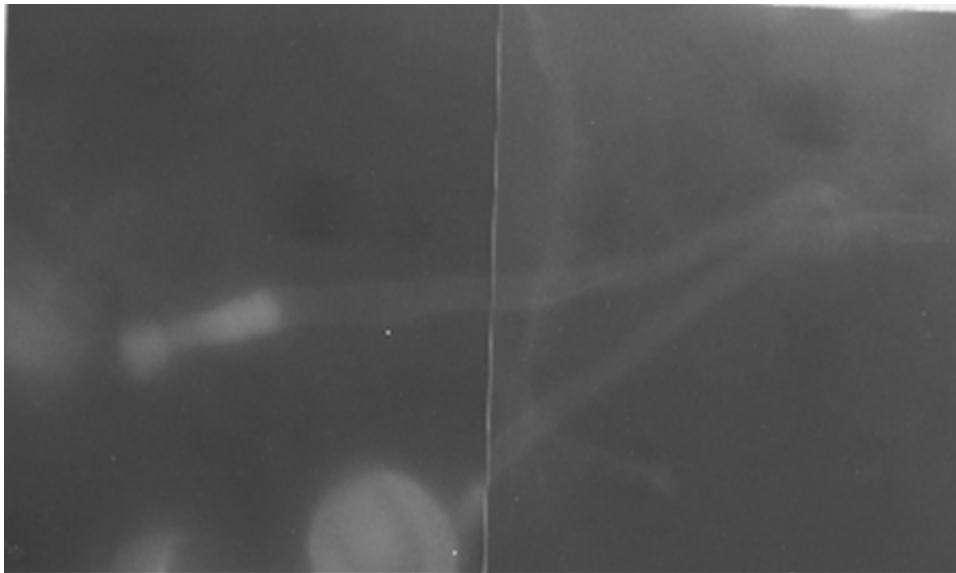


Fig. 4. Tube growth pattern of Kinnow pollen in the style of Minneola tangelo 24 hours after pollination (1040 microns).

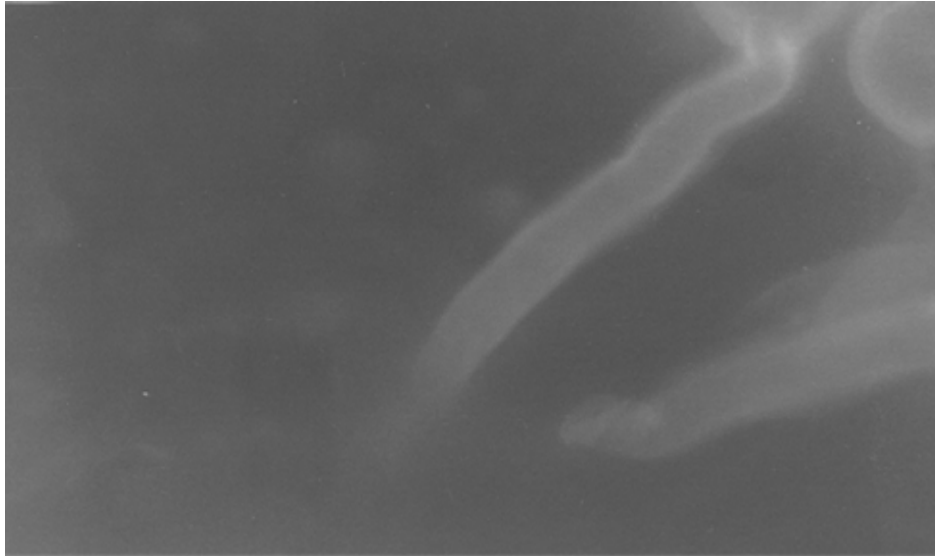


Fig. 5. Tube growth pattern of Kinnow pollen in the style of Samiola tangelo 24 hours after pollination (450 microns).

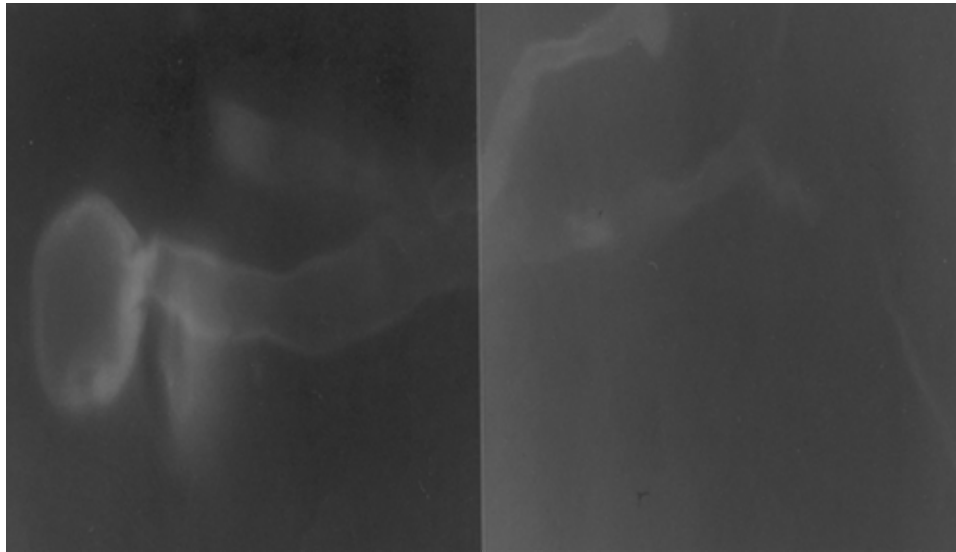


Fig. 6. Tube growth pattern of Kinnow pollen in the style of Orlando tangelo 24 hours after pollination (1100 microns).

Table 1. General combining ability of Mosambi, Kinnow and Duncan Grapefruit.

| Pollen source | Orlando | Minneola | Saminola | Pixie | Honey | FDT | Total (%) | GCA values |
|-------------------|-------------|------------------------------------|--------------|--------------|--------------|--------------|-----------|------------|
| Mosambi | 9.00 | 12.03 | <u>15.00</u> | 9.60 | 9.16 | <u>15.81</u> | 70.60 | 0.158 |
| Kinnow | 13.00 | 11.50 | 9.50 | 13.80 | <u>14.70</u> | 9.68 | 72.18 | 0.598 |
| Duncan grapefruit | 11.00 | 8.88 | 9.73 | <u>14.80</u> | 10.80 | 9.00 | 64.21 | -0.730 |
| Column total | 33.00 | 32.41 | 34.23 | 38.20 | 34.66 | 34.49 | GT | 206.99 |
| | | | | | | | GM | 11.49 |
| GCA values | -0.79 | -0.40 | -0.20 | 1.25 | 0.30 | -0.03 | | |
| Row Totals | % Fruit set | Crosses | | | | | | |
| 70.60 | 15.81 | Frost dancy tangerine x Mosambi | | | | | | |
| 72.18 | 14.70 | Honey mandarin x Kinnow | | | | | | |
| 64.21 | 14.80 | Pixie mandarin x Duncan grapefruit | | | | | | |

General combining ability (GCA): Results for general combining ability (GCA) are expressed as row totals in terms of percent fruit set in that row for a particular pollinizer for all hybrids used as female parents (Table 1), demonstrating the general combining ability of a pollen source with the individual experimental hybrid. Maximum fruit set (15.81%) was recorded in Frost dancy tangerine followed by Saminola (15.00%) when pollen from Mosambi was used for pollination. Mosambi stood at second position with a row total of (70.60%) after Kinnow with highest row total of 72.18%. Whereas the Mosambi yielded the highest fruit set percentage (15.81%, for Frost dancy tangerine and (15.00%) with Saminola tangelo, amongst all the citrus hybrids.

Pixie mandarin when fertilized by pollen from Duncan grapefruit resulted in 14.80% fruit set. Although, Kinnow as a male parent induced maximum overall fruit set (72.18%) by inducing more number of fruit on an average, but even then Honey x Kinnow held fourth position in terms of fruit set i.e., 14.70%. Duncan grapefruit was inferior of all the pollen parents as total fruit set (%) with the tested hybrids was 64.21%, as compared to 72.18% and 70.60% of Kinnow and Mosambi, respectively. Results revealed that the relationship between the over all row totals and per cent fruit set in a particular case is extinct i.e. a male parent resulting in maximum fruit set with one hybrid does not necessarily combine well with others to give highest fruit set percentage. It is also obvious from the results that Kinnow, generally proved to be a better pollen parent for all the tested hybrids. Kinnow excelled over the grapefruit by producing higher fruit set percentage in all the six hybrids tested, except that of Pixie mandarin, where grapefruit pollens induced more fruit set percentage, but the difference was negligible. Similarly, Kinnow excelled over Mosambi as a pollen source, except for the cross of Frost dancy tangerine and Saminola tangelo with Mosambi. So, Kinnow generally has more combining ability than Mosambi and Duncan grapefruit with the hybrids.

Specific combining ability (SCA): Results revealed that specific combining ability value was the highest for crosses between Frost dancy tangerine and Mosambi (3.937) followed by Saminola tangelo x Mosambi (3.377). Mosambi as pollinizer induced low fruit setting in Minneola tangelo, and resulted in SCA value of 0.437, while Orlando tangelo, Honey and Pixie mandarin resulted in negative values of SCA, indicating their poorest performance (Table 2).

Table 2. Specific combining ability of Mosambi, Kinnow and Duncan grapefruit.

| Crosses | Observed | Excepted | SCA Effect | SCA Values |
|---------------------------------|-----------------|--------------------|-------------------|-------------------|
| Mosambi | | | | |
| Frost dancy x Mosambi | 15.81 | -0.03+0.158+11.49 | =11.700 | 3.937 |
| Saminola x Mosambi | 15.00 | -0.20+0.158+11.49 | =11.448 | 3.377 |
| Minneola x Mosambi | 12.03 | -0.40+0.158+11.49 | =11.240 | 0.437 |
| Orlando x Mosambi | 9.00 | -0.79+0.158+11.49 | =10.850 | -2.133 |
| Honey x Mosambi | 9.16 | -0.30+0.158+11.49 | =11.940 | -2.183 |
| Pixie x Mosambi | 9.60 | 1.25+0.158+11.49 | =12.890 | -3.423 |
| Kinnow | | | | |
| Honey x Kinnow | 14.70 | 0.30+0.598+11.49 | =12.38 | 1.687 |
| Orlando x Kinnow | 13.00 | -0.79+0.598+11.49 | =12.87 | 1.407 |
| Minneola x Kinnow | 11.50 | -0.40+0.598+11.49 | =11.68 | 0.347 |
| Pixie x Kinnow | 13.80 | 1.25+0.598+11.49 | =13.33 | -0.630 |
| Frost dancy x Kinnow | 9.68 | -0.03+0.598+11.49 | =12.05 | -1.963 |
| Saminola x Kinnow | 9.50 | -0.20+0.598+11.49 | =11.88 | -2.213 |
| Duncan grapefruit | | | | |
| Pixie x Duncan grapefruit | 14.80 | 1.09-0.745+10.78 | =11.125 | 2.675 |
| Orlando x Duncan grapefruit | 11.00 | -0.77-0.745+10.78 | =9.265 | 0.735 |
| Honey x Duncan grapefruit | 10.80 | -0.25-0.745+10.78 | =10.285 | 0.515 |
| Minneola x Duncan grapefruit | 8.88 | -0.370-0.745+10.78 | =9.665 | -0.780 |
| Saminola x Duncan grapefruit | 9.73 | -0.15-0.745+10.78 | =9.885 | -1.155 |
| Frost dancy x Duncan grapefruit | 9.00 | -0.06-0.745+10.78 | =9.975 | -1.975 |

Honey mandarin performed the best in the group followed by Orlando tangelo in terms of fruit set extent with Kinnow as a pollinizer. Saminola tangelo showed least SCA value (-2.213), comparable to Frost dancy tangerine (-1.963) and Minneola tangelo (-0.630). It can be concluded that Kinnow generated the best value as a male parent with Honey mandarin and Orlando tangelo, while the performance of Kinnow with other hybrids was inconsistent (Table 2).

The cross between Pixie mandarin and Duncan grapefruit, yielded 13.80% fruit set with SCA value of 2.675. Specific combining ability values were negative for Frost dancy tangerine, Saminola tangelo and Minneola tangelo with Duncan grapefruit indicating as an inefficient pollinizer for these hybrids.

It is evident from the data that Kinnow expressed generally a better combining ability than Mosambi and grapefruit as a male parent. Crossing behaviour of Duncan grapefruit with Orlando tangelo and Saminola tangelo was quite different for both the tangelos, although in both the tangelos Duncan grapefruit is a mother parent. Close relationship can be considered as only explanation because in some rearrangement of genes in hybrids might create disturbance like self-incompatibility among varieties in the same spp. The results regarding artificial crosses in this study has been corroborated by Brown and Krezdorn (1969) and De Lange (1979) who found that hand pollination did not always accurately reflect a cultivar's effectiveness as a pollinizer in commercial plantings. So the inconsistency of data may be due to the factor by hand pollen application of Kinnow, Mosambi and Duncan grapefruit.

Crossing relationship between distantly related materials is not always encouraging. The closer genetic relationship thus could be advanced as a logical reason for this crossing relationship. It's strange that Pixie mandarin presented discouraging results for combination as such the plea of genetic relationship did not hold true in this case. In view

of such a situation it appears that some other factors are also involved, which can not be anticipated presently; so these studies suggest that another study should be organized to identify the genetic relationship of the hybrid cultivars because in some cases a single gene mutation might create disturbance like self-incompatibility among varieties in the same specie.

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