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FRUIT SETTING PATTERNS AND GERMINATION OF F_1 SEEDS IN SOME INTRA-SPECIFIC CROSSES OF MANDARIN (C*itrus reticulata* Blanco)

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ABSTRACT

The study was aimed to assess the compatibility of intra-specific mandarin crosses and germination of F_1 seeds under sub-tropical conditions of Punjab (India). Experiment was conducted at PAU, Ludhiana (Punjab) during 2014 and 2015. Six hundred crosses were made in each of four type crosses *viz*., 'Daisy x Kinnow', 'Kinnow x Daisy', 'W. Murcott x Kinnow' and 'Kinnow x W. Murcott'. After harvest of mature fruits from each cross, the F_1 seeds were sown under protected conditions. Results showed that the mean fruit set percentage was maximum (15.49) in 'W. Murcott x Kinnow' cross but was at par with 'Kinnow x W. Murcott' cross (14.16) and significantly higher than other crosses. Mean minimum fruit drop (31.87%) was observed in 'Kinnow x W. Murcott' which was at par with 'Kinnow x Daisy' and 'W. Murcott x Kinnow' crosses (32.29 and 34.79% fruit drop, respectively). The mean normal seed number fruit^{-1} was maximum (21.21) in fruits from 'Kinnow x Daisy' cross but was at par with 'Kinnow x W. Murcott' cross (18.01). Maximum number of seedlings seed^{-1} (1.17) emerged from 'W. Murcott' as female parent when 'Kinnow' was used as male parent which was at par with other crosses, except 'Daisy x Kinnow' which recorded single seeding seed^{-1}. The results revealed clear-cut difference in physiological mechanism controlling these parameters among these intra-specific crosses in mandarin.

Key words: Citrus, emasculation, fruit set, hybrid, germination, kinnow

INTRODUCTION

Citrus is an important crop widely cultivated in subtropical and tropical zones of the world. In India, occupies an area of 1.1 million ha with an annual production of 11.3 million tonnes (NHB, 2015). The natural and commercially cultivated citrus include sweet oranges, mandarins, grapefruits, lemons and limes. However, citrus cultivation is confined to a few varieties only. The monoculture of few promising cultivars in India has resulted in high risks associated with the outbreaks of new and more severe diseases and pests (Singh *et al*., 2016). The lack of knowledge on physiological and genetic mechanism controlling the compatibility of cultivars raises major obstacles in formulating the citrus breeding strategies.

Introduction of kinnow to India from USA in 20th century was an encouraging step for citrus industry. The introduction of a few new mandarin hybrids like Daisy and W. Murcott with varying harvest period boosted citrus industry in India. The fruitfulness of cultivars varies according to the prevailing pollination and environmental conditions in orchard. The inability of pollen to germinate on stigma is one of the reasons for unfruitfulness (Gul and Ahmad, 2006). Self-incompatibility is one of the important reasons of unfruitfulness in mandarin hybrids (Ahmed *et al.*, 2007).

The information on fruit setting behaviour and germination of F_1 seeds of different intra-specific mandarin crosses is lacking which limits crop improvement in mandarins grown under subtropical conditions. Flowering in plants is a genetically controlled phenomenon and is greatly influenced by the environment conditions and agronomic practices. The flowering and fruit load in citrus are influenced by the cultivar, plant age and environmental conditions; and only 1% flowers become mature fruit. Irregular flowering, low fruit setting and more fruit drop results in low economic yield in mandarin (Domingo *et al.*, 2007). Some endogenous factors are also responsible for low flowering response to inductive conditions. Fruit buds on previous summer shoots and buds at apical positions produce more flowers than the older or lateral buds (Valiente and Albrigo, 2004).

It is essential to understand the relationship between flowering, fruit setting and fruit drop in closely-related commercial cultivars. The identification of superior accessions and asexual propagation for use in crop improvement strategies may help in improving citrus fruit quality while retaining the essential characters of variability. Hence, the knowledge of cross compatibility among genotypes is pre-requisite to strengthen crop improvement programmes. In view of the importance of cross compatibility in developing new promising scion hybrids, the present study was aimed to assess compatibility of various citrus varieties and germination of F_1 seeds from the resultant crosses.

MATERIALS AND METHODS

The present experiment was conducted at the College Orchard, PAU, Ludhiana (India) during 2014 and 2015. The study included four types of intra-specific crosses in mandarin *viz*., 'Daisy x Kinnow', 'Kinnow x Daisy', 'W. Murcott x Kinnow' and 'Kinnow x W. Murcott'. A total of 600 flowers were emasculated and pollinated every year in each possible type of cross during 2014 and 2015. Each treatment was replicated six times, with 100 flowers comprising one replication. All the package of practices was followed as per PAU recommendation for the raising citrus nursery plants.

Emasculation and controlled pollination

The emasculation of flowers was performed in the morning hours (10.00 am to 12.00 noon) during the second fortnight of March 2014 and 2015 carefully with forceps to avoid any injury of the stigma. All previously opened flowers and small, immature buds were removed manually. Emasculated flowers were bagged in butter paper bags to avoid pollination by foreign pollen.

Pollination of emasculated flower buds was done on the same day between 1.00 pm and 3.00 pm with fresh pollen. Pollen was applied to flower stigmas with camel hair brush and then bagged. Each flower was labeled to avoid any mixing. Fruit set and fruit drop were recorded for each cross after removing the bag from pollinated flower. Seed germination in each cross was recorded after sowing the seeds in polythene bags of 11 x 8.5 cm size under polyhouse and seedlings emerging form each seed were recorded. Vigorous seeds were categorized as normal seeds whereas poor vigour seeds were categorized as rudimentary seeds.

Statistical analysis

The experiment was laid out as per the standard procedure meant for completely randomized design (Gomez and Gomez, 2010). The critical difference at 5% level of probability was computed so as to compare the statistical significance of various treatments. Analysis of variance was conducted for various quantitative traits using SAS (Statistical Analysis System) 9.3 version software.

RESULTS AND DISCUSSION

The mean fruit set was maximum in 'W. Murcott x Kinnow' cross (15.49 %) which was at par with mean fruit set in 'Kinnow x W. Murcott' cross (14.16 %) but significantly higher than other crosses (Table 1). The lowest mean fruit set (9.58 %) was observed in 'Kinnow x Daisy' cross which was at

Table 1: Fruit set and fruit drop in intra-specific crosses of mandarin

F_1 parentage	Fruit set (%)			Fruit drop (%)		
(F x M)	2014	2015	Mean	2014	2015	mean
Daisy x Kinnow	9.66^{bc}	13.30^{c}	11.48^{c}	37.93^{ab}	42.50^{a}	40.21^{a}
Kinnow x Daisy	7.50^{d}	11.66^{cd}	9.58^{cd}	28.88^{cd}	35.71^{b}	32.29^{bc}
W. Murcott x Kinnow	11.33^{a}	19.66^{a}	15.49^{a}	38.23^{a}	31.35^{cd}	34.79^{b}
Kinnow x W. Murcott	9.83^{b}	18.50^{ab}	14 .16^{ab}	32.22^{c}	31.53^{bc}	31.87^{bcd}
Mean	9.58	15.78	12.68	34.31	35.27	34.79
$LSD_{0.05}$	1.37	3.38	2.66	3.95	4.48	3.81

Different alphabets show significant difference and same alphabets show non-significant difference among genotypes

par with 'Daisy x Kinnow' (11.48%). Similar trend was observed during both the years (2014 and 2015). The results revealed higher fruit set in Kinnow when 'W. Murcott' used as a pollen parent as compared to 'Daisy' used as pollen parent. Furthermore, more fruits were set in 'W. Murcott' as compared to 'Daisy' when 'Kinnow' pollen was used in crossing. This may be due to higher pollen viability in 'Kinnow' as compared to 'W. Murcott'. However, 'Kinnow' showed lesser fruit setting with 'Daisy' as a pollen source. It has been reported (Domingo *et al*., 2007) that the activation of genetic programs regulating early growth and fruit set in seeded citrus fruit depends on pollination and hence of bloom quality whereas, in seedless cultivars the initiation of these programs appears to be linked to the development.

The maximum mean fruit drop (40.21%) was in 'Daisy x Kinnow' cross which was significantly higher than all other crosses. However, mean minimum fruit drop (31.87%) was observed in cross 'Kinnow x W. Murcott' which was at par with 'Kinnow x Daisy' and 'W. Murcott x Kinnow' recording 32.29 and 34.79% fruit set, respectively. Different trends of fruit drop were observed during both the years of study. During 2014, maximum fruit drop (38.23%) was noticed in 'W. Murcott x Kinnow' cross which was at par with 'Daisy x Kinnow' cross (37.93%) but significantly higher than 'Kinnow x W. Murcott' and Kinnow x Daisy' crosses which recorded 32.22 and 28.88% fruit drop, respectively. However, in 2015 maximum fruit drop (42.50%) was observed in 'Daisy x Kinnow' cross which was statistically higher than in all other crosses. Minimum fruit drop of 31.35% was recorded in 'W. Murcott x Kinnow' cross.

The results showed that 'Daisy' and 'W. Murcott' cross had higher fruit drop when 'Kinnow' was used as a pollen parent. However, 'Kinnow' recorded lower fruit drop when 'Daisy' or 'W. Murcott' were used as pollen parent (Table 1). The control of fruit growth and abscission is affected by genetic, metabolic and environmental factors. Citrus species usually produce large number of flowers over the year. Domingo *et al.* (2007) reported that sweet orange (*Citrus sinensis*) may develop more than 2.5 lakh flowers tree^{-1} in a bloom season and only less than 1% becomes mature fruit. The varietal compatibility plays an important role in fruit retention in addition to the environmental factors. In citrus, two successive waves of fruit drop occur. Flower and ovary abscission starts first and thereafter fruitlets are abscised affecting the fruit setting percentage in citrus

Table 2: Seed germination in intra-specific crosses of mandarin

F_1 parentage	Period for 50% germination (days)			Period for 100% germination (days)		
(F x M)	2014	2015	Mean	2014	2015	Mean
Daisy x Kinnow	50.00^{a}	54.67^{a}	52.33^{a}	91.00^{a}	85.33^{a}	88.17^{a}
Kinnow x Daisy	40.00^{c}	43.67^{bc}	41.83^{bc}	77.67^{b}	74.67^{bc}	76.17^{b}
W. Murcott x Kinnow	33.00^{d}	41.00^{bcd}	37.00^{bcd}	61.00^{d}	77.00^{b}	69.00^{bcd}
Kinnow x W. Murcott	47.00^{ab}	46.00^{b}	46.50^{ab}	73.00^{bc}	72.67^{bcd}	72.83^{bc}
Mean	42.50	46.33	44.42	75.67	77.42	76.54
$LSD_{0.05}$	6.58	5.13	6.55	10.74	4.82	8.28

Different alphabets show significant difference and same alphabets show non-significant difference among genotypes

(Rattanpal *et al*., 2009). The process of fruit abscission in citrus is very complex and regulated by hormones besides, a strong relationship between the source and sink affect fruit drop in citrus (Bangerth, 2000; Bonghi *et al*., 2000).

Seed germination

The mean maximum number of days for 50% seedling emergence (52.33) were taken by the seeds from 'Daisy x Kinnow' cross, which was significantly higher than all other crosses (Table 2). However, the mean minimum days for 50% seedling emergence (37) were taken by the seeds from 'W. Murcott x Kinnow' cross. Similar trend of seedlings emergence was observed during both the years.

The mean seed germination of normal seeds was maximum (80.95%) in seeds from 'W. Murcott x Kinnow' cross which was at par with 'Kinnow x W. Murcott' cross (71.31%) but significantly higher than other two crosses (Table 3). Mean minimum seed germination (56.66%) was recorded in 'Kinnow x Daisy' cross. Trend for seed germination was similar during both the years, except in case of 'W. Murcott x Kinnow' cross where significantly higher mean seed germination (88.44%) was recorded as compared to all other crosses in 2014. However, the mean seed germination of rudimentary seeds was maximum (44.0%) in seeds from 'Daisy x Kinnow' cross which was at par 'Kinnow x Daisy' cross (43.84%) and significantly higher than other two crosses. Mean minimum seed germination (32.9%) of rudimentary seeds was recorded in 'Kinnow x W. Murcott' cross. The rudimentary seed germination trend was almost similar in both the years of study. Mean maximum seed germination (68.8%) was recorded in 'W. Murcott x Kinnow' cross which was significantly higher than in other crosses, followed by 59.34 and 54.04% in 'Kinnow x W. Murcott' and Daisy x Kinnow' crosses, respectively.

The seed germination was higher in 'Kinnow' when 'W. Murcott' used as a pollen parent as compared to 'Daisy' as pollen parent. Further, 'W. Murcott' seeds had significantly higher germination when 'Kinnow' was used as a pollen parent. However, in rudimentary seeds 'Kinnow' and 'Daisy' seeds recorded significantly higher germination as compared to 'W. Murcott' seeds. This may be due to the presence of bold seeds in 'W Murcott x Kinnow' and 'Kinnow x W. Murcott' crosses. The germination duration of F_1 seeds from 'Daisy x Kinnow' was significantly higher which contributes to the lower seed germination. The rapid depletion of carbohydrates and protein in seeds might be responsible for the loss of seed vigour and hence germination percentage.

Statistically significant differences were observed in mean germination of different crosses suggesting important distention in mechanism controlling germination of seeds of different hybrids. Differences in seed germination of citrus seeds among sour orange and grapefruit have already been reported earlier by Orbovic *et al*. (2013). Early emergence of seedlings also contributes to the total germination percent in these crosses. Although 'Daisy' is an early maturing mandarin cultivar yet the total germination was higher in 'W. Murcott' when it was used as female parent with 'Kinnow' as pollen source. The germination of rudimentary seeds of 'Daisy' as a female parent was comparatively higher than other crosses because the rudimentary seeds in 'Daisy' get less number

Table 3: Germination of different types of seeds extracted from F_1 fruits of different intra-specific crosses in mandarin

F_1 parentage (F x M)	Normal seed germination (%)			Rudimentary seed germination (%)			Seed germination (%)		
	2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
Daisy x Kinnow	65.78^{bc}	53.48^{c}	59.63^{c}	43.78^{ab}	44.22^{a}	44.00^{a}	57.07^{cd}	51.01^{ab}	54.04^{bc}
Kinnow x Daisy	61.79^{cd}	51.53^{cd}	56.66^{cd}	47.23^{a}	40.44^{b}	43.84^{ab}	60.50^{bc}	47.13^{cd}	53.82^{bcd}
W. Murcott x Kinnow	88.44^{a}	73.45^{a}	80.95^{a}	38.60^{c}	37.02^{bc}	37.81^{c}	84.71^{a}	52.88^{a}	68.80^{a}
Kinnow x W. Murcott	73.44^{b}	69.18^{ab}	71.31^{ab}	30.57^{d}	35.23^{cd}	32.90^{cd}	70.22^{b}	48.46^{c}	59.34^{b}
Mean	72.36	61.91	67.14	40.04	39.23	39.64	68.13	49.87	59.00
$LSD_{0.05}$	10.18	9.55	11.17	6.28	3.44	5.34	10.72	2.23	7.01

Variable alphabets show significant difference and same alphabets show non-significant difference among crosses

Table 4: Seed number per fruit in F_1 seeds of different intra-specific crosses of mandarin

F_1 parentage (F x M)	Normal seeds fruit^{-1} (No.) 2014	2015	Mean	Rudimentary seeds fruit^{-1} (No.) 2014	2015	Mean	Total seeds fruit^{-1} (No.) 2014	2015	Mean
Daisy x Kinnow	12.66^{cd}	12.30^{cd}	12.48^{d}	9.33^{a}	4.47^{a}	6.90^{a}	21.99^{bc}	16.76^{bcd}	19.38^{bc}
Kinnow x Daisy	22.65^{a}	19.78^{a}	21.21^{a}	2.34^{b}	4.75^{ab}	3.54^{b}	24.99^{a}	24.52^{a}	24.76^{a}
W. Murcott x Kinnow	21.42^{ab}	14.60^{bc}	18.01^{ab}	1.61^{bc}	2.67^{cd}	2.14^{bc}	23.03^{ab}	17.27^{bc}	20.15^{b}
Kinnow x W. Murcott	15.72^{c}	17.29^{ab}	16.51^{bc}	1.15^{bcd}	2.73^{c}	1.94^{bcd}	16.87^{d}	20.02^{b}	18.45^{bcd}
Mean	18.11	15.99	17.05	3.61	3.65	3.63	21.72	19.64	20.68
LSD $_{(0.05)}$	4.09	2.81	3.63	3.33	0.96	2.29	3.00	3.08	2.80

Variable alphabets show significant difference and same alphabets show non-significant difference among the crosses

of days to mature and hence higher germination. Physiological studies and seed behaviour are very important because the preservation of seed vigour in citrus. It has been reported that seed vigour affects the overall viability and germination percentage in different hybrids (Orbovic *et al*., 2013).

Number of seeds per fruit

The seeds were extracted from mature fruits from all the crosses and data pertaining to number of seedlings per seed and number of F_1 seed per fruit were recorded (Table 4). The mean normal seed number fruit^{-1} was maximum (21.21) in fruits from 'Kinnow x Daisy' cross which was at par with mean normal seed number fruit^{-1} (18.01) in 'Kinnow x W. Murcott' cross and significantly higher than other two crosses. Significantly lower mean normal seed number fruit^{-1} (12.48) was recorded in 'Daisy x Kinnow' cross. The trends were similar in both the years where 'Kinnow x Daisy' cross yielded significantly higher seed number and 'Daisy x Kinnow' cross yielded lowest seed number fruit^{-1}. However, the trend was reverse as far as rudimentary seeds were concerned. Significantly, higher mean rudimentary seed number fruit^{-1} (6.90) was recorded in 'Daisy x Kinnow', followed by 3.54 in 'Kinnow x Daisy' cross. The lowest mean number of rudimentary seeds (1.94) was recorded in 'Kinnow x W. Murcott'. The trend of mean rudimentary seed number was similar in both the years. The mean total seed number fruit^{-1} was maximum (24.76) in seeds from 'Kinnow x Daisy' cross which was significantly higher than other crosses. However, the lowest total mean seed number fruit^{-1} (18.45) was recorded in 'Kinnow x W. Murcott' cross.

Higher normal seeds number fruit^{-1} was observed when 'Kinnow' was used as female parent and 'Daisy' as a pollen source. However, the number of normal seeds in 'Kinnow' decreased when 'W. Murcott' was used as pollen source. As far as rudimentary seed number was concerned, 'Daisy' recorded maximum number of normal seeds fruit^{-1} when 'Kinnow' was used as a male parent. The total seed number was influenced by the pollen source. 'Kinnow' yielded significantly higher seed number when 'Daisy' was used as pollen parent and it recorded minimum seed number when 'W. Murcott' was used as pollen source. This may be due to the pollen viability of source parent. The pollen viability of 'W. Murcott' was comparatively lower than that of 'Daisy' and 'Kinnow'.

Number of seedlings per seed

The maximum number of seedlings seed^{-1} emerged from 'W. Murcott' (1.17) as female parent when 'Kinnow' was used as male parent which was at par with other crosses, except 'Daisy x Kinnow' which had 1 seedling seed^{-1} (Table 5). The trends were almost similar in both the years of study. Since 'Daisy' mandarin is mono-embryonic in nature, only single seedling emerged from a seed in 'Daisy' when used as a female parent in 'Daisy x Kinnow' hybridization; whereas all other parents were highly poly-embryonic and hence recorded more

Table 5: Seedling number per seed in F_1 seeds of intra-specific crosses of mandarin

F_1 parentage (F x M)	Number of seedlings seeds^{-1} 2014	2015	Mean
Daisy x Kinnow	1.00^{d}	1.00^{d}	1.00^{c}
Kinnow x Daisy	1.09^{bc}	1.15^{ab}	1.12^{ab}
W. Murcott x Kinnow	1.16^{a}	1.17^{a}	1.17^{a}
Kinnow x W. Murcott	1.11^{ab}	1.12^{abc}	1.12^{ab}
Mean	1.09	1.11	1.10
LSD $_{(0.05)}$	0.06	0.07	0.07

Variable alphabets within same column show significant difference and same alphabets non-significant difference among crosses number of seedlings seed^{-1}.

The study revealed that 'Kinnow' expressed generally a better compatibility as male parent with 'W. Murcott' and vice-versa. This might be due to self-incompatibility in 'W. Murcott' due to some arrangement of genes in hybrids. However, lower fruit setting in 'Daisy' with 'Kinnow' as a male revealed lower compatibility. Further, hand pollination did not always accurately reflect a cultivar's effectiveness as pollinizer in commercial cultivation. In our study good fruit setting was observed in all the crosses which indicate the cross-compatibility of these mandarin cultivars. Similarly, higher F_1 seed germination in 'Kinnow' fruits when 'W. Murcott' used as a pollen parent as compared to when 'Daisy' as pollen parent indicates their better compatibility. The study revealed good cross compatibility and seed germination of commercial cultivars under sub-tropics. However, the significant differences in fruit setting, seed germination, fruit drop and number of seedlings per seed in different intra-specific cross suggests an important distinction in mechanism controlling these parameters in different hybrids.

Conclusion: The study indicated higher cross compatibility among commercial mandarin genotypes used in this study. Better compatibility had been observed between 'Kinnow' and 'W. Murcott' when 'W. Murcott' used as a pollen source. Significant differences were observed in mean germination percentage of different crosses suggesting distention in mechanism controlling germination of seeds of different hybrids. The study also revealed that early emergence of seedlings and vigorousness of F_1 seeds contribute to higher total germination of F_1 seeds.

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