

GROWTH REGULATORS APPLICATION AFFECTS VEGETATIVE AND REPRODUCTIVE BEHAVIOUR OF 'BLOOD RED' SWEET ORANGE

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Abstract

Two plant growth regulators (GA₃ and 2, 4-D) were exogenously applied in different concentrations alone and their combinations on Blood Red sweet orange trees during full bloom. The experiment was performed at the Experimental Fruit Garden Sq # 9 Institute of Horticultural Sciences, University of Agriculture, Faisalabad; laid out according to RCBD with 13 treatments including control replicated thrice. A single tree was taken as treatment unit. Vegetative and reproductive behaviours of the treated trees were studied to evaluate the effect of exogenous application of plant growth regulators on leaf age, vegetative growth and final fruit set. The leaf drop was significantly reduced by all treatments compared with control. The mixture treatments at all concentrations retained maximum spring leaves ranging between 38.5% (20 mg L⁻¹) - 58.38% (45 mg L⁻¹). The control trees had negligible number of spring leaves (3.09%). Number of leaves per flush, flush length, number of male flowers and flower drop intensity had also significant differences among treatments but no linear trend could be determined. Bud drop, hermaphrodite flowers, fruit on old shoots and fruit on current shoots were not significantly affected by the treatments. The final fruit set was significantly affected by all GA₃ treatments individually as well as in mixture with maximum fruit set of 32.32% in 45 mg L⁻¹ GA₃ treated trees compared with control. Fruit yield, in terms of number of fruit per tree as well as kg per tree was significantly affected by the treatments compared with control. There was positive correlation between number of fruit and weight of fruit per tree.

Introduction

Low productivity of sweet oranges in Punjab, Pakistan is an obstacle in its large scale cultivation, lagging them behind in citrus industry as growers are inclined to 'Kinnow' mandarin cultivation turning industry to monoculture. Among various reasons of low productivity of sweet oranges, shedding of leaves during winter has been considered a serious threat to their production (Chaudhary, 1992). New growth flushes in spring, the main flush in subtropical areas, develop mostly without presence of old leaves which affects growth and vigour, trees striving for their existence resulting in poor yields. Presently, in Pakistan, the per hectare average production of citrus including Kinnow is about 10 MT which is far less than world average yield of 30 t ha⁻¹ (Anon., 1993). Annually, 1.7 MMT citrus is produced from a total area of 185 thousand hectares in Pakistan (Anon., 2005). Today, Pakistan stands at 13th and 10th position respectively among top citrus producing and exporting countries of the world (Anon., 2005).

Exogenously applied plant growth regulators have modified growth and development in wide range of plants. Growth of stems and other organs is promoted by GA and results from enhanced cell division, increased carbohydrate hydrolysis, and increased cell wall plasticity (Sachs, 1961; Salisbury & Ross, 1978; Boyle *et al.*, 1994). A lot of work has

been done on exogenous application of growth regulators to citrus for improvement of yield and quality of fruit (Hield *et al.*, 1965; Saleem *et al.*, 2007; Saleem *et al.*, 2008a) and these have also been used to alter flowering, fruit set, fruit thinning and fruit abscission (Malik *et al.*, 1993; El-Otmani *et al.*, 1995; Berhow, 2000; Saleem *et al.*, 2008b), but work on leaf retention is lacking. However exogenous application of gibberellins (GA) and cytokinins have been used to enhance postharvest life of Easter lilies leaves (Ranwala & Miller, 1999; Ranwala *et al.*, 2000; Whitman *et al.*, 2001).

Keeping in view the importance of leaf retention in citrus and role of plant growth regulators in delaying leaf senescence, this study was designed to evaluate the effect of exogenous growth regulators on over wintering citrus leaves age and its impact on reproductive behaviour including fruit set, fruit drop and fruit yield of 'Blood Red' sweet orange. Overall objective of the experiment was to increase the productivity of sweet oranges.

Materials and Methods

The study was conducted on 15 years old sweet orange (*Citrus sinensis* Osbeck L.) cv. Blood Red trees growing at the Experimental Fruit Garden Sq No. 9, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Punjab, Pakistan. The experimental trees were spaced at about 7 m x 7 m, grafted on rough lemon rootstock, growing under similar agroclimatic conditions and received same cultural practices during the period of investigation (Saleem *et al.*, 2004).

Before start of the experiment, the trees were evaluated for uniformity of growth, fruit yield potential and possible disease incidence. Thirty nine uniform trees with no apparent disease incidence were selected for the experiment in the form of a block. The soil analysis was done for checking the fertility status of the soil by collecting samples at different depths from four sites of the experimental orchard. The composite soil samples from under the tree in the orchard were also collected from four sites. Soil analysis was done according to standard methods available at Farm Advisory Centre of Fauji Fertilizer Company (FAC-FFC) Lab., Jhang. On four sides of each tree 4-5 cm thick branches were selected for data collection and average of these four branches was taken as a single replication data. A Randomized Complete Block Design (RCBD) was followed with three replications and a single tree was selected as a treatment unit.

Exogenous growth regulator application: Efficacy of a wide range of concentrations of GA₃ and 2, 4-D and their mixtures was tested on 'Blood Red' sweet oranges to improve the production and quality during the years 2005-06. Aqueous solution of all the treatments was prepared and sprayed on whole trees to run off during full bloom according to method earlier described by Saleem *et al.*, (2007).

T ₀	Control	T ₇	30 mg L ⁻¹ 2, 4-D
T ₁	20 mg L ⁻¹ GA ₃	T ₈	45 mg L ⁻¹ 2, 4-D
T ₂	25 mg L ⁻¹ GA ₃	T ₉	GA ₃ + 2, 4-D 20 mg L ⁻¹ each
T ₃	30 mg L ⁻¹ GA ₃	T ₁₀	GA ₃ + 2, 4-D 25 mg L ⁻¹ each
T ₄	45 mg L ⁻¹ GA ₃	T ₁₁	GA ₃ + 2, 4-D 30 mg L ⁻¹ each
T ₅	20 mg L ⁻¹ 2, 4-D	T ₁₂	GA ₃ + 2, 4-D 45 mg L ⁻¹ each
T ₆	25 mg L ⁻¹ 2, 4-D		

(G= GA₃, G₁= 10 mg L⁻¹, G₂= 20 mg L⁻¹, G₃= 30 mg L⁻¹, G₄= 45 mg L⁻¹, D= 2, 4-D, D₁= 10 mg L⁻¹, D₂= 20 mg L⁻¹, D₃= 30 mg L⁻¹, D₄= 45)

Vegetative behaviour: The vegetative behaviour of the experimental trees was assessed by recording number of leaves per flush and length of flush and leaf age. Five twigs of equal size were tagged on each branch already tagged for data collection, for recording the number of leaves per flush and length of the flush. The number of leaves per flush was recorded when the flush was fully developed and there was no sign of new leaf. When the number of leaves was counted, length of shoot was also measured with the help of scale and average of these flushes (for twenty twigs) was worked out.

For leaf age, 20 flushes per tree were tagged to record the data, the number of leaves per shoot was recorded at the time two months after their emergence (when the leaf expansion was completed) and then were counted in mid February from the same 20 shoots. The leaf age was expressed as % of leaves retained. The words shoot and flush meant for the new small branches with new leaves in spring flush.

Reproductive behaviour: The data regarding reproductive behaviour including total number of buds, bud drop (%), intensity of male and hermaphrodite flowers (%), flower drop (%), fruit set (%) fruit drop and fruit yield were collected on 20 twigs per tree. Number of flowers at the time of full bloom was counted and later on flower drop was monitored at an interval of three days. Total droppage between the period of full anthesis and initial fruit set was taken as flower drop. The percentage was calculated on the basis of total number of flowers per twig. The male flowers were counted on weekly basis after anthesis started and these were counted up to fruit set. The percentage was counted on the basis of total number of flower buds per branch. The fruit set was counted when maximum fruit was seen without style and petal fall was ended. The fruit set percentage was calculated on the basis of total number of buds per branch on 4th April. Periodic fruit drop at different intervals was calculated on the basis of fruit set, while total fruit drop was calculated at the time of total fruit count. Total number of fruit per tree was counted on 18th October and was taken as fruit yield per tree and total weight was also measured by balance.

Statistical analysis: The response of experimental trees to different treatments was evaluated by statistical analysis of data using the computer software MSTAT-C (Freed & Scott, 1986), while DMR test was used to compare the differences among the treatment means at 5% probability level.

Results and Discussion

The soil analysis indicated that the soil of orchard area was alkaline in nature (8.13-8.50 pH), with most of the elements in optimum level except nitrogen and phosphorus which were in deficient range.

Vegetative behaviour: The intensity of leaves on a flush was significantly affected by treatments compared with control with maximum number of leaves (13.76) in trees treated with 30 mg L⁻¹ mixture of GA₃ + 2, 4-D, while minimum in control trees having 4.11 leaves per flush (Table 1). Shoot length was significantly increased by all the exogenous application of growth regulators with maximum shoot length (7.39 cm) again in trees treated with 30 mg L⁻¹ mixture of GA₃ + 2, 4-D. The minimum size of a flush (2.30 cm) was recorded in control trees (Table 1). The leaf age was also significantly increased by different treatments of plant growth regulators compared with control (Fig. 1). Maximum leaf retention (58.38%) was observed in trees sprayed with 45 mg L⁻¹ GA₃ + 2, 4-D mixture, similar to all other levels of mixture treatments. The control trees dropped most of their leaves as minimum retention (3.09%) was recorded in these trees (Table 1).

Number of leaves in a shoot, length of shoot and leaf retention determines the health and vigour of a tree. The mixture treatments improved the vegetative growth with number of leaves, leaf age and shoot length, although all the results were not in the same pattern. The importance of endogenous growth regulators in affecting many growth and morphogenetic processes has been well documented (Jacobs, 1968). The 2, 4-D is a synthetic phenoxy compound which delays the development of abscission layer in different organs like leaves and fruits especially in fruit crops. Growth of stems and other organs is promoted by GA and results from enhanced cell division, increased carbohydrate hydrolysis, and increased cell wall plasticity (Sachs, 1961; Salisbury & Ross, 1978; Boyle *et al.*, 1994). The life of citrus leaves vary greatly, depending on the climate and overall vigour of the tree. The retention of about 50% old leaves before coming active growth season (spring) was encouraging as previous year foliage in citrus undoubtedly plays a critical role in provision of photosynthate during the emergence of the spring flush at least prior to full expansion of new leaves (Kriedmann, 1969; Moss, 1972; Shimizu *et al.*, 1978). Physiological activity of 50 leaves is required for the production of one orange (Mennone, 2004). The extension in leaf age and improvement in vegetative growth might be due to exogenous application of growth regulators (Ranwala & Miller, 1999; Ranwala *et al.*, 2000 Whitman *et al.*, 2001).

Reproductive behaviour: Reproductive behaviour of the trees in response to exogenous application of different plant growth regulators at different levels was inconsistency (Table 1). The experimental trees were significantly alike in bud drop and hermaphrodite flowers intensity. However, there were significantly different number of male flowers among different treatments compared with control, with maximum male flowers (5.01%) in 30 mg L⁻¹ GA₃ treated trees, statistically similar to those of 20 mg L⁻¹ 2, 4-D treated trees (3.85%), while minimum male flowers (1.43%) were observed in G₄ (45 mg L⁻¹ GA₃) statistically similar to all other treatments. Flower drop was also significantly different among treatments compared with control with maximum flower drop of 90.40% in 25 mg L⁻¹ GA₃ treated trees, while minimum flower drop (68.68%) was recorded in trees treated with G₂ + D₂. Flower drop intensity was significantly decreased by different treatments compared with control with maximum flower drop of 90.40% in G₂, statistically similar to control. Minimum flower drop of 57.16% was noted in G₄, statistically different from all other treatments. All the experimental trees had statistically similar number of fruit on old as well as current shoots compared with control. However, fruit set was significantly improved by all treatments compared with control (Fig. 1). Maximum fruit set of 32.32% was observed in trees treated with 45 mg L⁻¹ GA₃ statistically similar to all other GA₃ treatments, while minimum fruit set was observed in control trees.

The periodic fruit drop from fruit set to harvest in response to exogenous application of different concentrations of GA₃, 2, 4-D alone and in combination at full bloom (Fig. 2). Most of the treatments had similar pattern of fruit drop compared with control, while 20 mg L⁻¹ GA₃ + 2, 4-D, 10 mg L⁻¹ GA₃+2, 4-D and 20 mg L⁻¹ GA₃ had different pattern of fruit drop. On 10th April, one week after fruit set (WAFS), maximum fruit drop (61.51%) was noted in 20 mg L⁻¹ 2, 4-D compared with control and significantly different from all other treatments, while minimum fruit drop (20.40%) was observed in G₂ compared with control, statistically similar to 20 mg L⁻¹ GA₃ + 2, 4-D (24.30%) and 10 mg L⁻¹ GA₃ + 2, 4-D (23.45%). On 17th April (2 WAFS) all the treatments had non significant differences in intensity of fruit drop (13.77-23.73%), except 20 mg L⁻¹ GA₃+2, 4-D having maximum value of fruit drop (49.07%)

compared with control followed by 20 mg L⁻¹ 2, 4-D (36.96%) and 45 mg L⁻¹ GA₃ + 2, 4-D (31.94%). On 1st May (4 WAFS), maximum fruit drop was noted in 10 mg L⁻¹ GA₃ + 2, 4-D (45.74%) compared with control statistically different from all other treatments, while minimum fruit drop (14.89%) was noted in G4 + D4 compared with control statistically similar to 20 mg L⁻¹ 2, 4-D (16.89%), 45 mg L⁻¹ 2, 4-D (18.79%) and 20 mg L⁻¹ GA₃ + 2, 4-D (21.12%). All other treatments had near about similar fruit drop ranging between 25.24 - 37.39 %. On 31 May (8 WAFS), 30 mg L⁻¹ GA₃ had maximum value (6.66%) of fruit drop, statistically different from all other treatments, while all other treatments had non significant differences of fruit drop intensity which ranged between 0.40–.35%. Again on 18 October (26 WAFS), the pattern was similar as on 31st May with maximum fruit drop (4.48%) in G₃ compared with control and minimum (0%) in G₂ + D₂ treated trees. The overall range of fruit drop during this period was 0–3.34%.

Regarding total fruit drop, there were significant differences in treatments with maximum total fruit drop (97.38%) being in G₄, while minimum total fruit drop (91.84%) was recorded in G₃ compared with 95.76% in control. Most of the treatments behaved statistically alike and clear trend could not be established (Table 1).

Fruit yield, in terms of number of fruit per tree as well as kg per tree, was significantly affected by the treatments compared with control (Fig. 3). Maximum number of fruit was harvested from G₄ treatment, statistically similar to all mixture treatments, while minimum fruit were recorded from D₄ treatment, statistically similar to control trees. Fruit weight is affected by number of fruit (Fig. 4), the correlation between fruit weight and yield in terms of number of fruit per tree. Positive correlation was found between number of fruit and fruit weight per tree (Fig. 4).

Economic yields depend primarily on adequate flowering and subsequent fruit set from those flowers (Albrigo & Galan Sauco, 2004), which ultimately reach to maturity by surviving through different waves of fruit drop. The results pertaining to reproductive behaviour did not indicate any uniform behaviour with respect to intensity of maleness in flowers and flower drop, however the fruit set was significantly affected by GA₃ application. Maleness of flowers is not desirable character in sweet oranges as most of the flowers are hermaphrodite. The differences in maleness and flower drop among treatments might be attributed to intensity of flower bud formation and individual behaviour of trees. According to previous reports most commercially important citrus cultivars bloom prolifically producing as many as 100,000 – 200,000 flowers on a mature tree: however fewer than 1-2% of these flowers will produce harvestable fruit (Erickson & Brannaman, 1960). In citrus, major flower drop occurs from early flowering stages until 2 or 3 weeks after full bloom. Ovule fertilization usually plays a major role in subsequent retention and 2.5 to 3 months after full bloom a major fruitlet drop often occurs due to carbohydrate competition (Sanz *et al.*, 1987; Guardiola, 1997).

The results regarding fruit set revealed pronounced involvement of GA₃ in fruit setting but not 2, 4-D (Fig. 1). When 2, 4- D applied to Valencia orange trees at full bloom, these sprays delayed blossom drop 8-10 weeks or more but did not increase fruit set (Stewart & Klotz, 1947). The involvement of hormones in fruit setting has been reported by many worker but the exogenously applied hormones like GA₃ and 2, 4-D had inconsistent effect on fruit setting as Davies & Albrigo (1994) reported that GA₃ application @ 30 mg L⁻¹ did not improve fruit set of most of the citrus cultivars. Similarly, the GA₃ application to Washington Navel sweet orange at the time of full bloom had no effect on fruit setting (Moss, 1972). On the other hand, it is commercial practice in California to spray GA₃ for the improvement of fruit set in citrus.

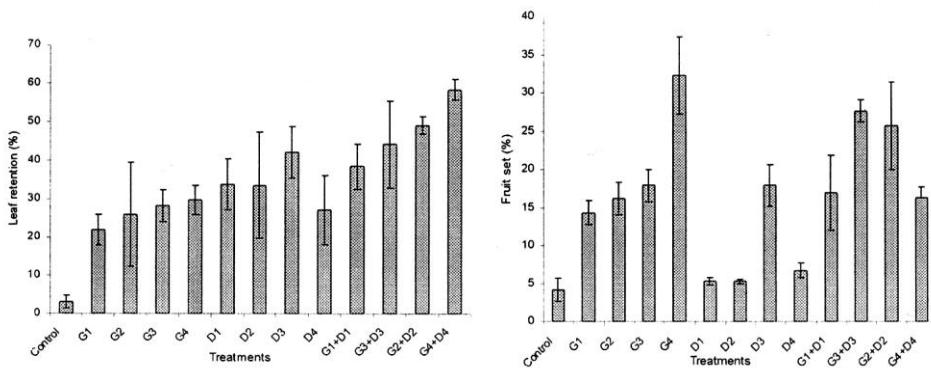


Fig. 1. Leaf retention (left) and Fruit set (right) of Blood Red sweet orange in relation to different plant growth regulators treatments (\pm s.e.)

G= GA₃, G₁= 10 mg L⁻¹, G₂= 20 mg L⁻¹, G₃= 30 mg L⁻¹, G₄= 45 mg L⁻¹, D= 2, 4-D, D₁= 10 mg L⁻¹, D₂= 20 mg L⁻¹, D₃= 30 mg L⁻¹, D₄= 45 mg L⁻¹,

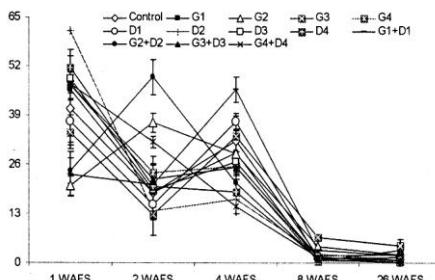


Fig. 2. Periodic fruit drop of Blood Red sweet orange in relation to different plant growth regulators treatments (\pm s.e.)

G= GA₃, G₁= 10 mg L⁻¹, G₂= 20 mg L⁻¹, G₃= 30 mg L⁻¹, G₄= 45 mg L⁻¹, D= 2, 4-D, D₁= 10 mg L⁻¹, D₂= 20 mg L⁻¹, D₃= 30 mg L⁻¹, D₄= 45, WAFS= Weeks after fruit set

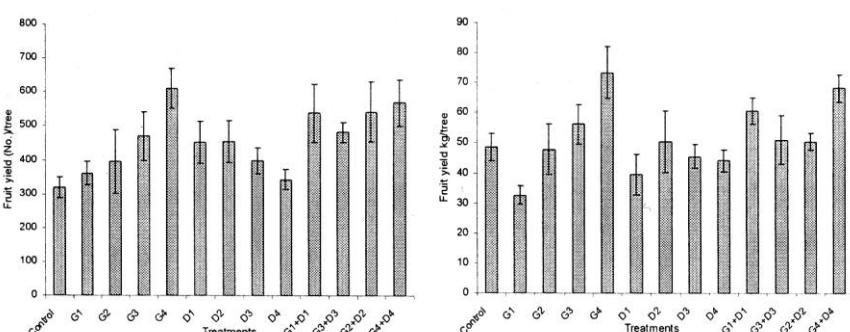


Fig. 3. Fruit yield/tree (Fruit no. left; kg right) of Blood Red sweet orange in relation to different plant growth regulators treatments (\pm s.e.)

G= GA₃, G₁= 10 mg L⁻¹, G₂= 20 mg L⁻¹, G₃= 30 mg L⁻¹, G₄= 45 mg L⁻¹, D= 2, 4-D, D₁= 10 mg L⁻¹, D₂= 20 mg L⁻¹, D₃= 30 mg L⁻¹, D₄= 45 mg L⁻¹.

Shoots older than one year are less likely to have floral buds induction (Guardiola, 1981). Although this effect is important to determine the inductive conditions needed for different aged shoots in citrus, the proportion of flowers on each aged shoot can not be extrapolated to the whole tree level unless the proportions of shoots of different ages are known (Albrigo & Galan Sauco, 2004). In our studies most of the fruit was produced on current year shoots with small percentage on old shoots ranging from 0.28% to 6.97% (Table 1) albeit there was no effect of treatments.

Fruit drop is natural fruit load management phenomenon and a large quantity of fruit usually drops during initial stages of fruit setting and development. The results regarding patterns of fruit drop in 'Blood Red' sweet orange revealed that only 3-7.43% of fruit set reached to maturity level which was about 0.5 to 2% of total flower buds count. Although most of the treatments had the same pattern of fruit drop as previously reported (Erickson & Brannaman, 1960), however, the total fruit drop at the time of final fruit count was significantly different compared with control. Some of the treatments retarded fruit drop during the first two weeks compared with other treatments, but the final fruit retention was found approximately same. Initial fruit set, subsequent fruit drop and ultimately fruit yield are affected by interaction of several environmental and physiological factors. The results are in accordance with previous reports as natural fruit drop of 'Hamlin' orange was significantly delayed due to 2, 4-D and GA₃ applications (Medeiros *et al.*, 2000). It was reported by Gomez-Cadenas *et al.*, (2000) that exogenous application of gibberellins had no effect on abscission in citrus. The GA₃ (30 mg L⁻¹) application to 'Washington Navel' sweet orange at flower opening retarded the fruit shedding initially, but the final result was not significantly different from control (Moss, 1972). Fruit production was increased by application of GA₃, whether alone or in combination with 2, 4-D. However, there was no effect of 2, 4-D on final fruit production of Blood Red sweet orange. Already, we observed no effect of 2, 4-D on fruit set as compared with control, and the fruit drop remained at par among different treatments with total yield unaffected (Table 1). Since GA₃ caused more fruit to set, it had good impact on final fruit harvest, while 2, 4-D neither improved fruit set nor affected final fruit harvest. Previous reports support our findings as increased fruit set and production resulted from GA applications in low yielding Clementine mandarin trees, but the production was not increased under adequate cross pollination (Soost & Burnett, 1961). There was positive correlation between fruit weight and fruit number in our study which is supported by findings of Blanke & Bower (1991), who stated that small sized fruit of Valencia sweet orange trees were due to a large number of fruit per tree, which might be attributed to inefficient fruit photosynthesis, causing excessive respiration by large number of fruit. Similarly, Thakur & Chandel (2004) reported that fruit size and weight of kiwifruit were significantly increased by thinning of fruit.

Other effects: Exogenous application of plant growth regulators, 2, 4-D alone as well as in mixtures at all levels caused downward curling of leaf margins of Blood Red sweet orange when sprayed at full bloom with new leaves (Fig. 5). However, it did not influence the tree performance. The curling of leaves by the application of 2, 4- D during the period of full bloom in our experimental trees (Fig. 5) confirms earlier report of Stewart & Klotz (1947) about leaf curling of Valencia orange at higher concentrations (30 and 60 mg L⁻¹). The leaf curling in our case was almost in the same intensity with all concentrations of 2, 4- D. However, in the long run leaf curling had no negative effect on growth and vigour of trees.

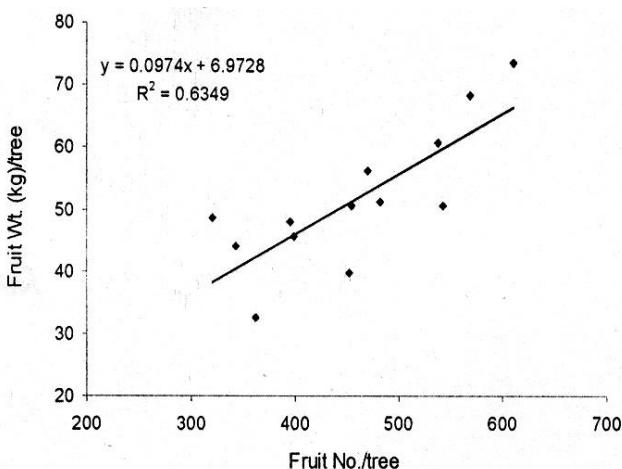


Fig. 4. Correlation between number of fruit and fruit weight per tree of Blood Red sweet orange.



Fig. 5. Downward curling of young leaves of Blood Red sweet orange in response to aqueous sprays at different concentrations of 2, 4-D ranging from 20 mg L^{-1} – 45 mg L^{-1} . (Photograph 2 days after spray).

Conclusion

From the investigations it is clear that with exact dose and time of exogenous application of plant growth regulators, vegetative and reproductive behaviour can be positively manipulated. The growth regulators especially GA_3 has pronounced effect on increasing the fruit set in citrus.

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