DOI : 10.5958/0974-0112.2017.00070.6

Indian J. Hort. 74(3), September 2017: 351-356

Effect of high density planting systems on physiological and biochemical status of rejuvenated mango plants of cv. Amrapali

Amit Raj, V.B. Patel*, Ravindra Kumar, Kalyan Barman, R.B. Verma, Sashikant and S.K. Pathak**

Department of Horticulture (Fruit & Fruit Technology), Bihar Agricultural College, Sabour, Bhagalpur 813210

ABSTRACT

Mango trees grown under high density planting systems show a gradual decline in yield after 11-12 years due to overcrowding of canopies. To find out the effect of high density planting systems on physio-biochemical parameters of mango, an experiment was conducted on an old senile, rejuvenated high density cv. Amrapali orchard over two consecutive years. High density planting systems showed significant effect on physiological, biochemical and nutritional parameters of plants. Among the different planting systems, plants under cluster planting system recorded the highest leaf area (156.68 cm^2), transpiration rate (3.77 m mol^{-1} $m^{-2}s^{-1}$); and N (1.28%) P (0.20 %) and K (0.54%) contents. The maximum leaf relative water content (87.63%) was noted in plants under square planting system, while, the photosynthetic rate (8.36 µmol CO_2 m^{-2} s^{-1}) was highest in hedge-row planting system. The stomatal conductance (0.17 µmol CO_2 m^{-2} s^{-1}) was the highest in plants under double-hedge row planting system. The highest internal carbon concentration was recorded in paired planting system, while the maximum chlorophyll '*a*' (1.50 mg g^{-1}), chlorophyll '*b*' (0.65 mg mg g^{-1}) and total chlorophyll (2.02 mg g^{-1}) contents were found in plants under square planting system. The highest total phenolics (53.09 mg g^{-1}) content was recorded under paired planting system. The micronutrient contents in plants, *viz*. Cu, Zn, Fe and Mn also differed among different planting systems.

Key words: High density planting, mango tree, micronutrient, physiology.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most popular and choicest fruit crops of tropical origin. It belongs to the family Anacardiaceae and originated in Indo-Myanmar region. Owing to its delicious taste, appealing aroma, majestic appearance and rich nutritional value, this fruit occupies a superior position in the national and international markets. The fruit is an excellent overall nutritional source, rich in a variety of phytochemicals, vitamins and minerals. Mango is rich in antioxidant vitamins A, C and E. Besides that, vitamin B_6 (pyridoxine), vitamin K, other B vitamins (thiamine, riboflavin, niacin and pantothenic acid), essential nutrients (potassium, calcium, copper and iron) and 17 amino acids are also present in good levels. In mango, the concept of high density planting has gained momentum after the development of cultivar Amrapali (Dashehari × Neelum) a distinctly dwarf and regular-bearing hybrid. However, the mango plants grown under different high density planting systems show progressive decline in yield as well as fruit quality after 10-11 years of planting owing to overlapping/ intermingling of branches, poor light interception, low photosynthetic rate and high relative humidity within the tree canopy (Asrey *et al*., 2). Therefore, the time and severity of pruning, which is adopted for rejuvenation of mango orchards not only alter the physiological status of the plant but also modify its biochemical attributes, which is manifested by its flowering, bearing behavior and yield pattern. Previously several studies have been conducted on the effect of pruning in mango trees in relation to its micro-climate for better light penetrance, fruit set and yield performance. The physiological, biochemical and nutritional parameters in these earlier studies have received only little attention. However, beyond the routine information, there is an increasing interest among the researchers to know the effect of high density planting and pruning on plant physiological, biochemical and nutritional parameters of mango trees after rejuvenation. Hence, the present investigation was carried out.

MATERIALS AND METHODS

The field experiment was conducted in All India Coordinated Research Project (Fruit), Sabour, the permanent experimental site of Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India (25°15’40” North, 87°2’42” East, elevation 46 m). The experiment was conducted on old senile orchard of cv. Amrapali mango planted in different planting systems. The

---

*Corresponding author’s present address: Div. of Fruits & Horticultural Technology, ICAR-IARI, New Delhi; E-mail: patelvb7@gmail.com

**Instt. of Agril. Sci. Banaras Hindu University, Varanasi 221005 experiment was carried out on rejuvenated plants that were planted in 1992 and become over-croweded and unproductive in 2008. Hence, rejuvenation pruning was done in July, 2009 with the help of mechanized pruner at a uniform height of 1.5 m. Thinning was done during 2010 and 2011 as per recommended practices. All the trees were maintained under uniform cultural practices. The experiment was laid out in randomized block design (RBD) with five treatments (*i.e.* planting systems) and five replications. The different plantings systems taken as treatments were (i) Square system (1,600 plants/ ha, and 9 plants/ plot), (ii) Hedge row system (2,670 plants/ ha and 15 plants/ plot), (iii) Double-hedge-row system (3,556 plants/ ha and 20 plants/ plot) 4. Paired planting (2,133 plants/ ha and 12 plants/ plot), and (v) Cluster planting (2,844 plants/ ha and 16 plants/ plot). Normal planting distance of 2.5 × 2.5 m and half-normal distance of 1.25 × 1.25 m were followed in this experiment. Normal plant spacing of 2.5 m × 2.5 m was maintained in square system of planting, while in hedge row system, spacing of 1.25 m × 2.5 m (P-P × R-R) was maintained. In double-hedge row system, 2 rows of hedge were planted at half-normal distance (1.25 m × 1.25 m). In case of paired planting, pairs of two plants at the distance of 1.25 m × 1.25 m were maintained. Cluster planting system accommodated cluster of 4 plants at 1.25 m × 1.25 m apart. Net area under each plot was 56.25 m^2 and total experimental area was 2,406 m^2.

Leaf relative water content (RWC) was determined following method of Weatherly (15). Leaf area was measured by laser leaf area meter (CI-203). Photosynthetic rate, stomatal conductance, transpiration rate and internal carbon ratio were recorded using photosynthesis system infra-red gas analyzer (LI-COR 6400 XT). Chlorophyll contents (chlorophyll *a*, chlorophyll *b* and total chlorophyll) of the leaves were analyzed at the time of fruiting (Barnes *et al*., 3), total phenols by Malik and Singh (5) using spectrophotometer (HALODB-20S UV-vis double beam spectrophotometer, Australia).

The macro- and micro-nutrients were estimated from shoot samples of mango at vegetative stage. For determination of total nitrogen content, 500 mg of oven dried sample was taken in a 250 ml Kelplus tubes. To this, 3.9 g digestion mixture containing 3.5 g K_2SO_4 and 0.4 g $CuSO_4$ was added and digested with 12 ml concentrated H_2SO_4 on the digestion unit till light green colour appeared. The digested sample was distilled and total nitrogen was determined by Kelplus KES 20LR. For estimation of phosphorous, potassium and micronutrients, tissue samples were wet digested with a diacid mixture of concentrated nitric acid and per chloric acid (9:4 v/v). Fifteen ml of diacid mixture was added to 500 mg of the prepared samples and pre-digested at a temperature of 100°C for an hour and at 250°C until the solution turned colourless and the volume reduced to 2-3 ml. The digested material was then diluted and filtered through Whatman No. 1 filter paper and volume was adjusted up to 100 ml. The filtrate obtained was used for the estimation of phosphorus, potassium, zinc, copper, manganese and iron.

Phosphorus was estimated by vando-molybdate colour reaction method. Per cent transmittance at 420 nm was measured with the help of spectrophotometer. Potassium (K) content was determined by a microprocessor based flame photometer using specific filter (K filter) and LPG flame. The different standard K solutions (5, 10, 20 to 100 ppm) were atomized to standardize the instrument. Suitably diluted di-acid digest was then fed to an atomizer capillary tube and concentration was directly read on the display monitor. Per cent K content was calculated on dry weight basis. Micronutrients (Cu, Fe, Mn and Zn) were determined by atomic absorption spectrophotometer (ECIL, Hyderabad) directly from the diacid digest using an air-acetylene flame. The concentrations of Cu, Fe, Mn and Zn were measured at 386.0 nm (lamp current 7 mA), 22.6 nm (lamp current 3 mA), 403.1 (lamp current 5 mA) and 213.9 nm (lamp current 5 mA) wavelengths, respectively. Final concentration was calculated in ppm by multiplying the concentration with suitable dilution factor. The data generated from the experiment were analyzed by following factorial RBD (Panse and Sukhatme, 8).

RESULTS AND DISCUSSION

After one year of rejuvenation, plants under high density planting system was recorded a profuse vegetative growth in all the directions. Therefore, in second year thinning was done as per recommended practices. Data was recorded in third year of rejuvenation, which showed quite interesting changes in plant physiology and nutrients.

High density planting systems after rejuvenation did not exert significant differences in leaf area and relative water content of leaf (Table 1). This might be due to single variety taken in all the planting systems. However, some differences were recorded among the treatments in respect to leaf area. Plants under cluster planting were recorded the highest leaf area (156.68 cm^2), which was at par with hedge row planting system (149.24 cm^2) and paired planting system (146.24 cm^2). The minimum leaf area (137.20 cm^2) was recorded in plants of square planting system. A possible reason for differences in the leaf area may be attributed to water stress as a result of competition of plants for air and water in closed

Table 1. Effect of high density planting systems on leaf area and leaf relative water content of Amrapali mango plants after rejuvenation.

Treatment	Leaf area (cm^2)	Relative water content (%)	Yield (t/ ha)
Square planting	137.32	87.63	11.05
Hedge-row planting	149.24	85.28	18.21
Double-hedge row planting	141.6	86.44	13.54
Paired planting	146.24	76.41	17.52
Cluster planting	156.68	75.5	20.88
CD at 5%	11.89	13.03	2.44

spacing. It is generally seen that leaf area of plant is increased in the plants which are under water limitation though this is not true with all the plants and may vary from species to species. However, in our investigation, similar results were found in cluster planting system, where higher leaf area was recorded because of water stress to plant, due to competition of plant for air and water because of narrow spacing.

The leaf relative water content was recorded highest (87.63%) in square planting system, which was closely followed by double-hedge row (86.44%) and hedge row planting (85.28%). The minimum leaf relative water content was found in cluster planting (75.50%), due to large leaf surface area and narrow spacing. The higher leaf relative water content in square planting system might be due to smaller leaf surface area, which lost minimum moisture, resulted into maximum water retention in the leaves. This result is in accordance with the findings of Singh *et al*. (13). In this study, planting systems having less number of plants/ plot (square planting system) showed lower leaf area and higher leaf water content, while, planting system having more number of plants/ plot exhibited the larger leaf area with minimum relative water content.

Rejuvenation of high density mago plants showed significant differences in rates of photosynthesis (Table 2). The maximum photosynthetic rate (8.36 μmol CO_2 $m^{-2}s^{-1}$) was recorded in plants of hedge-row planting system, which was at par with cluster planting (8.23 μmol $CO_2 m^{-2}s^{-1}$), followed by square planting system (7.47 μmol $CO_2 m^{-2}s^{-1}$) and double-hedge row planting system (7.26 μmol $CO_2 m^{-2}s^{-1}$). In this study, high density planting systems showed significant effect on photosynthetic rate, stomatal conductance, transpiration rate and internal carbon concentration. A possible reason for high photosynthetic rate in hedge-row planting system might be due to the maximum non-flowering branches present in plants of hedge-row planting system. The non-flowering branches were reported to be high in internal carbon concentration, which are responsible for higher photosynthetic rate than flowering branches (Shivashankara *et al.*, 11).

The significant variation in stomatal conductance was also found among plants under high density planting systems after rejuvenation. Among the treatments, maximum stomatal conductance (0.17 μmol m^{-2} s^{-1}) was recorded in double-hedge row planting system, which was at par with hedge-row planting system (0.14 μmol m^{-2} s^{-1}), whereas, the minimum stomatal conductance (0.10 μmol m^{-2} s^{-1}) was recorded in plants of square planting system. Stomatal conductance and net CO_2 assimilation rate in fruit species depend upon conditions such as solar irradiance, leaf temperature, soil and plant water status and mineral nutrition. Higher stomatal conductance in double-hedge row system plants and lower stomatal conductance in square planting system might be due to the fact that stomatal conductance always found higher in less vigorous plant and lower in the more vigorous plants (Murti and Upreti, 6). In double-hedge row planting system, the maximum number of plants was accommodated per unit area as a result; the height of plant increased more rather than plant vigour. Similarly, stomatal conductance was

Table 2. Effect of high density planting systems on photosynthetic rate, stomatal conductance, transpiration rate and internal carbon concentration of Amrapali mango after rejuvenation.

Treatment	Photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (μmol m^{-2} s^{-1})	Transpiration rate ($mmol^{-1}$ $m^{-2}s^{-1}$)	Internal CO_2 conc. (ppm)
Square planting	7.47	0.10	1.51	237.52
Hedge-row planting	8.36	0.14	2.25	243.52
Double-hedge row planting	7.26	0.17	1.81	224.55
Paired planting	6.05	0.12	1.89	245.38
Cluster planting	8.23	0.13	2.37	244.95
CD at 5%	1.22	0.017	0.33	16.50

lower in square planting system owing to vigorous growth of plant due to wider spacing than rest of the planting system.

The rate of transpiration also varied significantly among the planting systems. The higher transpiration rate was recorded in plants of cluster planting (2.37 mmol $m^{-2}s^{-1}$) and hedge row planting system (2.25 mmol $m^{-2}s^{-1}$). Plants under square planting showed the lowest transpiration rate (1.51 mmol $m^{-2}s^{-1}$) among the treatments. The higher transpiration rate in cluster planting might be attributed to maximum leaf area and lower leaf relative water content, which resulted into maximum transpiration of plant. Similarly, in square planting system, the minimum leaf area and higher leaf relative water content caused lowest transpiration from the leaf (Singh *et al.*, 13).

The internal carbon dioxide concentration was recorded maximum (245.38 ppm) in paired planting, while, it was minimum (224.55 ppm) in double-hedge row planting. The higher internal carbon dioxide concentration in paired planting system may be due to maximum light penetrance and low relative humidity within the tree, due to low canopy volume of the plant. This is an essential criterion for carbohydrate metabolism for higher fruit yield/ plant.

In the present study, the maximum chlorophyll '*a*' (1.50 mg g^{-1}) content was recorded in square planting, while, it was minimum (0.97 mg g^{-1}) in double-hedge row plants (Table 3). No significant difference in chlorophyll content was recorded between hedge-row, paired and cluster planting system plants. Among the treatments, highest chlorophyll '*b*' (0.65 mg g^{-1}) content was noted in plants of square planting system followed by hedge-row planting system (0.62 mg g^{-1}). The lowest chlorophyll '*b*' content was noticed in paired planting system (0.28 mg g^{-1}). The maximum total chlorophyll was recorded in square planting system (2.02 mg g^{-1}), which was at par with hedge row planting system (1.60 mg g^{-1}), while, it was minimum (1.21 mg g^{-1}) in double-hedge row planting system. The results depicted that plants under square planting system exhibited significantly higher content of chlorophyll '*a*', '*b*' and total chlorophyll than other treatments. Similar results were also reported by Abirami *et al.* (1). A positive correlation between vigour of mango plant and chlorophyll content was also reported by Pal *et al.* (7). Sharma and Singh (10) also reported that the value of total chlorophylls content was highest in leaves of vigorous un-pruned trees.

The total phenols content was estimated highest (53.09 mg g^{-1}) in plants of paired planting system, which did not differ significantly with square planting system (52.70 mg g^{-1}) (Table 3). The plants of double-hedgerow planting system showed lowest content of total phenols (41.08 mg g^{-1}) among the treatments. In present study, the highest total phenols content was found in paired planting system may be due to minimum canopy volume. Murti and Upreti (6) reported that phenols play a vital role in restricting vigour of plant and also confirmed that total phenols content in leaves showed significant inverse relationship with plant height and stem girth. In our study to similar results were also noticed. Higher total phenols content in the plants of paired planting was the possible cause of low vigorous growth, which resulted in minimum canopy volume and maximum canopy temperature within the plant, thus exhibiting maximum yield.

Table 3. Effect of high density planting systems on chlorophyll content of Amrapali after rejuvenation.

Treatment	Chlorophyll (mg g^{-1})			Total phenols
	Chl '*a*'	Chl. '*b*'	Total	(mg g^{-1})
Square planting	1.50	0.65	2.02	52.70
Hedge-row planting	1.25	0.62	1.60	49.97
Double-hedge row planting	0.97	0.35	1.21	41.08
Paired planting	1.04	0.28	1.26	53.09
Cluster planting	1.03	0.44	1.40	41.68
CD at 5%	0.20	0.07	0.33	8.46

High density planting systems after rejuvenation had significant effect on tissue nutrient content of mango (Table 4). Pruning leads to high mobilization of N, P and K nutrients in plants (Singh *et al.*, 14). In this experiment, highest nitrogen content was recorded in cluster planting (1.28%), which was at par with double-hedge row planting system (1.22%). The minimum nitrogen (N) content was recorded in square planting (1.04%). Nitrogen is the main growth manipulating nutrient. It is found higher in reproductive shoots. The nitrogen content differed among the planting systems. This difference may be due to more number of flowered shoots in cluster planting than square planting. Devi and Tyagi (4) also found similar results that total nitrogen content was higher in flowered shoots than in non-flowered shoots. The phosphorus (P) content did not differ significantly among the treatment. However, Ram (9) also found higher P contents in the leaves during on year/ during flower bud differentiation than in the off year/ vegetative stage. The highest potassium (K) content was also found in cluster planting system (0.54%), which was at par with treatment of hedge row (0.53%), paired planting (0.52%) and double-hedge row (0.51%). The lowest K was found in square planting (0.42%). The potassium is not directly involved in the metabolism of the plant that might be one of the important reasons that high

Table 4. Effect of high density planting systems on tissue nutrient status in Amrapali after rejuvenation.

Treatment	Macro-nutrient (%)			Micro-nutrient (ppm)			
	N	P	K	Fe	Cu	Mn	Zn
Square planting	1.04	0.19	0.42	271.92	15.19	258.81	70.25
Hedge-row planting	1.14	0.19	0.53	236.00	23.37	326.97	58.81
Double-hedge row planting	1.22	0.18	0.51	173.95	19.41	306.16	53.67
Paired planting	1.14	0.20	0.52	213.22	24.73	200.43	73.49
Cluster planting	1.28	0.20	0.54	255.59	21.6	229.14	64.72
CD at 5%	0.08	NS	0.10	36.97	2.76	38.17	12.32

density planting system did not show significant differences in potassium content in tissues. Similar results were also reported by Singh and Rathore (12).

In case of micronutrients content, all the treatments differed significantly in zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) contents in leaf. Pruning showed marked influence on Zn, Cu, Fe and Mn content. Among the treatments, the highest Fe content was recorded in square planting system (271.92 ppm), which was at par with cluster planting system (255.59 ppm). The lowest Fe content was found in double-hedge row planting system (173.95 ppm).Iron is one of the micronutrients needed in small quantities by the plants to produce chlorophyll. In square planting system, higher Fe content resulted into higher chlorophyll content in the leaves and it also supported the contrary findings in double-hedge row planting system. Copper (Cu) is one of the micronutrients needed in very small quantities by plants. Copper activates enzymes in plants, which are involved in lignin synthesis and it is essential in several enzyme systems. It is also required in the process of photosynthesis, plant respiration carbohydrate and protein metabolism. In this study, copper content was recorded highest in paired planting system (24.73 ppm), which might be responsible for higher fruit yield/ plant while, it was recorded lowest in square planting system (15.19 ppm). Manganese (Mn) is an essential plant mineral nutrient, playing a key role in several physiological processes. It enhances the photosynthetic efficiency and dry matter production; provide resistance to biotic stresses. The highest manganese (Mn) content was recorded in hedge row planting system (326.97 ppm), which was at par with double-hedge row planting system (306.16 ppm). This higher Mn content in hedge-row planting resulted in higher respiration photosynthetic rate (Table 2). The lowest Mn content (200.43 ppm) was noticed in paired planting system. The highest zinc (Zn) content was found in paired planting system (73.49 ppm), which was statistically at par with the square planting system (70.25 ppm). The lowest Zn content was found in double-hedge row planting system (53.67 ppm). Zinc is one of the essential micronutrients required for optimum crop growth. Zinc transported in the xylem tissues from the roots to the shoots. Translocation of zinc takes place from older leaves to the younger leaves during fruit development phase. Zinc act as precursor for auxin, regulate starch formation and proper root development, and help in formation of chlorophyll and carbohydrates, helps in biosynthesis of cytochrome that's why it is the possible reason that the highest zinc content was found in paired planting system, which turned in to highest number of fruits per plant in comparison to other planting systems. While, it recorded lowest in double hedge row planting system resulted into low fruit yield. The severe pruning of Amrapali mango, planted under different high density planting systems, affected physiological and biochemical parameters significantly. The change in the physiological state of the plants after pruning not only the result of age but also the systems of planting.

REFERENCES

1. Abirami, K., Singh, R. and Baskaran, V. 2011. Studies on the influence of seedling physiological parameters with vigour in some polyembryonic and monoembryonic mango genotypes. *Indian J. Hort.* **68**: 18-23.

2. Asrey, R., Patel, V.B., Barman, K. and Pal, R.K. 2013. Pruning affects fruit yield and postharvest quality in mango (*Mangifera indica* L.) cv. Amrapali. *Fruits*, **68**: 367-80.

3. Barns, J.D., Balaguer, L., Manrique, E., Elvira, S. and Davison, A.W. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophyll *a* and *b* in lichen and higher plants. *Env. Exp. Bot.* **32**: 83-100.

4. Devi, T.M. and Tyagi, D.N. 1991. Physiology of mango (*Mangifera indica* L.): Fractions of

carbohydrates, nitrogen and related enzymes in leaves of flowered and non-flowered shoots of mango. *Indian J. Pl. Physiol.* **34**: 30-36.

5. Malik, E.P. and Singh, M.B. 1980. *Plant Enzymology and Hittoenzymology* (1st Edn.), Kalyani Publishers, New Delhi, 286 p.
6. Murti, G.S.R. and Upreti, K.K. 2003. Endogenous hormones and phenols in rootstock seedlings of mango cultivars and their relationship with seedling vigour. *European J. Hort. Sci.* **68**: 2-7.
7. Pal, R.N., Srivastava, R.D., Singh, N.P. and Chadha, K.L. 1980. Chlorophylls, dry matter and leaf area in relation to vigour of mango rootstocks. *Indian J. Hort.* **38**: 9-15.
8. Panse, V.G. and Sukhatme, P.V. 1984. *Statistical Methods for Agricultural Workers* (3rd Edn.), ICAR, New Delhi, India.
9. Ram, S. 1999. Hormonal physiology of flowering in 'Dashehari' mango. *J. Appl. Hort.* **1**: 84-88.
10. Sharma, R.R. and Singh, R. 2006. Effect of pruning intensity on light penetration and leaf physiology in Amrapali mango trees under high density planting. *Trop. Sci.* **46**: 16-19.
11. Shivashankara, K.S. and Mathai, C.K. 2000. Inhibition of photosynthesis by flowering in mango (*Mangifera indica* L.): A study by gas exchange methods. *Scientia Hort.* **83**: 205-12.
12. Singh, P.N. and Rathore, V.S. 1983. Changes in moisture content, dry matter accumulation, chlorophyll and nutrient contents in mango malformation. *Indian J. Hort.* **40**: 21-25.
13. Singh, S.K., Sharma, R.R. and Shrivastav, M. 2009. Effect of pruning on morpho physiological parameter and microclimate under high density planting of mango (*Mangifera indica*). *Indian J. Agric. Sci.* **79**: 632-35.
14. Singh, S.K., Singh, S.K. and Sharma, R.R. 2010. Effects of pruning intensity on the biochemical status of shoot buds in three mango (*Mangifera indica* L.) cultivars planted at high density. *J. Hort. Sci Biotech.* **85**: 483-90.
15. Weatherly, P.E. 1950. Studies in water relation of cotton plants. I. Field measurement of water deficit in leaves. *New Physiologist*, **49**: 81-87.

Received : September, 2016; Revised : July, 2017;
Accepted : August, 2017