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Impact of rejuvenation on physiological and biochemical status of different mango cultivars under *sub-Himalayan Terai region* of West Bengal

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ABSTRACT

Twenty-three years old five mango cultivars viz. Amarapali, Mallika, Himsagar, Fazli and Langra were rejuvenated (severe pruning) 150 cm from the ground level. Total phenol content, peroxidase, poly phenol oxidase, total carbohydrates and total free amino acids as well as total chlorophyll content were measured in 2019-2020 and 2020-2021 seasons in shoot buds with few leaves. The aforesaid parameters were measured before rejuvenation as well as four months after regrowth of pruned plants of different mango cultivars. Total phenol content was recorded higher (35.19 mg/g) in Langra and minimum amount of total phenols (35.50 mg/g) was recorded in Mallika before rejuvenation of plants. Chlorophyll content was decreased after rejuvenation of plants of all five cultivars, cv. Himsagar (1.08 mg/g) recorded higher chlorophyll content before rejuvenated plants. The total carbohydrate was not significantly varied before and after the rejuvenation practice and it was highest with cv. Himsagar before (0.80%) and after (0.74%) the rejuvenation. Maximum amount of total amino acid was recorded with cv. Amrapali (1.31%) before the rejuvenation and after pruning it was recorded higher on cv. Himsagar (2.0 %). Negligible difference recorded for peroxidase activity, maximum amount of peroxidase activity (0.37min/g) was recorded in cv. Himsagar before and after the rejuvenation and minimum (0.30 min/g) quantity observed in before and after rejuvenation plants of Mallika. The changes in PPO activity due to rejuvenation, the maximum activity of catechol oxidase (0.72) and laccase (0.62) were recorded in Langra.

Keywords: *Chlorophyll content, Himsagar, Mango cultivars, Peroxidase, Poly phenol oxidase, Total carbohydrates, Total free amino acids, Total phenol content.*

Introduction

Mango is one of the most relished fruits in India and remains a unique, delicious crop; it has been part of Indian culture and religion since ancient time and thus, becomes '*National fruit*'. Because of its sweet peerless test and richness in phytochemical and nutrient content, it is called as the *King of Fruit*. Mango having good nutritional value as every 100 g of mango fruit contains 81.7 g water, 16 g carbohydrate, 0.7 g protein, 0.4g fat and 0.1 g fibres. It is rich in calcium, phosphorus, iron, magnesium, Vitamin-A, B and C and also anti-oxidants. A single fruit can provide up to 40 percent of daily dietary fiber needs. Mango fruits have various uses from unripe to ripen stages, unripe mangoes are used for making pickles, marmalade, amchur, tannin, soft drinks, etc. while fully ripe mangoes are used for pulp making, jam, squash, candy and *papads* etc.

High density planting of mango (5×5m or even low) is gaining popularity day by day for more productivity. After 10 to 12 years of fruiting, all three mango cultivars exhibited a decrease in fruit yield and quality when planted at high densities. This was caused by canopy factors like branch overlap or intermingling, poor light interception, low photosynthetic rates, and/or high relative humidity [1], which made the plants more vulnerable to pests and/or diseases. These conditions alter the physiology of individual shoots or entire trees, making them unhealthy in subsequent years. Rejuvenation is the process of pruning and after pruning management of the plants to make the productive by utilizing the existing root system, restoring the productive capacity of the fruit trees. The rejuvenation makes the plant manageable, easy for adoption of an appropriate package of practices, improving vigour and photosynthetic activity during fruit growth period might be augmented to increase yield [5]. Rejuvenation not only improves the vigour and yield but also alter the physiological and biochemical changes in mango cultivars [19]. Rejuvenation induce the stress in plants and it promote some reserves to hydrolyze and metabolites accumulation [10]. It will also the change flowering, fruiting and yield behaviour of plants.

Previously several studies has been conducted on the effect of rejuvenation on mango to it micro climate for better light penetration, improved the vigour, flowering, fruit set and yield performance. The physiological and bio chemical parameters in these earlier studies have received only limited information especially before and after rejuvenation of mango cultivars. However, beyond the earlier routine information, there is extra information provided among the researchers to know the

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physiological, biochemical and nutritional changes by rejuvenation of mango plants before and after rejuvenation and the impact of rejuvenation on trees. Hence, the present study was conducted.

Material and Methods

The field experiment was conducted on twenty-three years old five mango cultivars viz. Amrapali, Mallika, Himsagar, Fazli and Langra planted at 7 x 7m distance at the Instructional Farm of the Department of Pomology and Post Harvest Technology, Uttar Banga Krishi Viswavidyalaya under sub-Himalayan *Terai* region of West Bengal and crowed and unproductive plants were headed back at 150 cm above from the ground level. The different rejuvenated cultivars were considered as the treatments under this experiment. Hence rejuvenation done one set of plants were pruned during February, 2019 and another set were pruned during February, 2020. For heading back of old branches, a sharp slanting cut towards inner side was especially undertaken as to enhance sprouting of apical buds from periphery i.e. outer side of the branch as suggested by [9]. Immediately after pruning the cut portion of branches was pasted with copper oxychloride (10%) followed by cow dung to prevent the possible infection of diseases and to minimize the sap flow from pruned limbs and to accelerate healing of pruned apical portion. A recommended dose of fertilizer consisting 50 kg FYM and 0.7:0.4:0.8 kg NPK was applied to each tree in the month of June and same quantity of fertilizer applied in the month of october as a second dose [7]. The experiment was laid out in randomized block design (RBD) with five treatments (cultivars) and four replications.

Total phenol content, peroxidase, poly phenol oxidase (PPO), total carbohydrates and total free amino acids as well as total chlorophyll content were measured in 2019-2020 and 2020-2021 seasons in shoot buds with few leaves in five mango cultivars (Amarapali, Mallika, Himsagar, Fazli and Langra). To measure the total phenol content shoot tips [13] along with a pair of freshly emerged leaves were used. Foliar sample (approx. 500 mg) was homogenized in mortar by adding 80 per cent ethanol. It was then centrifuged at 10,000 rpm for 20 min. and the supernatant was filtered using filter paper Whatman No. 42. The residue was re-extracted (5 times) with 80 per cent ethanol and the supernatant collected were evaporated to dryness (60 °C) on a water bath. Residues were dissolved in 5 ml of distilled water from which about 0.2 ml was taken and total volume was made up to 3 ml with distilled water. To this, fresh Folin-Ciocalteau reagent (0.5 ml) was added. After 3 min., 2 ml of Na2 CO3 (20 %) solution was added to each tube, mixed thoroughly and placed on a hot water bath (58 °C) exactly for one min. It was then cooled to room temperature and absorbance (650 nm) was measured against blank.

To estimate the peroxidase used [16] method, A 20 percent homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from plant leaves, clarified by centrifugation and the supernatant was used for the assay. To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5ml of H_2O_2 was added and mixed. The change in absorbance was recorded in every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change by absorbance/minute at 430nm. Polyphenol oxidase (Catechol oxidase and laccase) activities were estimated simultaneously by the method as described by [6]. The enzyme extract was prepared by homogenizing 0.5g of plant tissue in 2.0ml of the extraction medium containing *tris* HCl, sorbitol and NaCl. The homogenate was centrifuged at 2000rpm for 10 minutes and the supernatant was used for the assay. Phosphate buffer (2.5ml) and 0.3ml of catechol solution were added in the cuvette and the spectrophotometer was set at 495nm. The enzyme extract (0.2ml) was added and the change in absorbance was recorded every 30 seconds up to 5 minutes by spectrophotometer.

One unit of catechol oxidase or laccase is defined as the amount of enzyme that transforms 1µmole of dihydro phenol to 1µmole of quinone per minute.

The activity of PPO was calculated by using the formula

Enzyme units in the sample = K × (ΔA/minute) where, K for catechol oxidase = 0.272

K for laccase = 0.242

Total carbohydrate estimation proposed by [17], 100mg of the leaf sample was taken into a boiling tube and hydrolyzed by keeping it in a boiling water bath for three hours with 5mL of 2.5 N-HCl and cooled to room temperature after that the sample was neutralized with solid sodium carbonate until the effervescence ceased. Final volume was made up to 100 ml and then centrifuged 10000 rpm for 20 minutes. The supernatant was collected and 0.5-1mL of aliquots was taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard. '0' serves as blank and volume to 1mL was made in all the tubes including the sample tubes by adding distilled water. Then 4mL of anthrone reagent was added and the samples were heated for eight minutes in a boiling water bath cooled rapidly and read the green to dark green colour at 630nm. A standard graph was drawn by plotting the concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph the amount of carbohydrate present in the sample was calculated.

$$\frac{\text{Amount of carbohydrate present in 100mg of the sample mg of glucose}}{\text{Volume of test sample}} = \times 100$$

The plant leaf sample 500mg was taken and ground it in a pestle and mortar with a small quantity of acid-washed sand. To this homogenate, 5 to 10 mL of 80 percent ethanol was added and filtered, filtrated and sample was saved and repeated the extraction twice with the residue and pool all the supernatants. The total free amino acid was determined as suggested by [17].

Total chlorophyll content was estimated spectrophotometrically at 645 and 663 nm according to [2]. 1g of leaf sample was taken and finely grinded in clean mortar and pestle with the addition of 20mL of 80 percent acetone. The sample was centrifuged at 5,000rpm for 5 min and the supernatant was transferred to a 100mL volumetric flask. The remaining residue was ground again with 20mL of 80 percent acetone, centrifuged and the sample solution was transferred to the same volumetric flask and the same procedure was repeated until the leaf residue turned to colorless. The mortar and pestle was thoroughly washed with 80 percent acetone and the clear washings in the volumetric flask was collected. The volume of the sample was made up to 100mL with 80% acetone and the absorbance of the sample solution was read at 645, 663 and 652nm against the solvent (80% acetone) blank. The amount of total chlorophyll content present in the extract was calculated using the equation

$$\text{mg Total Chlorophyll /g tissue} = 20.2\,(A645) + 8.02\,(A\,663) \times \frac{V}{1000 \times W}$$

Results and Discussion

Bio chemical properties were recorded before rejuvenation and after 120 days of rejuvenation of plants when profuse vegetative growth was observed all directions. Total phenol content was recorded higher (35.19 mg/g) in Langra (Table 1) which was statistically at per (35.13 mg/g) with Fazli, whereas, the minimum amount of total phenols (35.50 mg/g) was recorded in Mallika, before rejuvenation. The higher phenol content (35.13 mg/g) is responsible for less number of leaves (45.54) in Himsagar after rejuvenation (Fig.1). It indicating that vital role in restricting the vigour of plant as suggested by [14, 20]. [15] reported that the total phenol content of rejuvenated mango cv. Amrapali was varied between (41.08-53.09mg/g) which was similar with the report of present experiment. The enzyme phenylalanine ammonia lyase, present in higher plants, is known to convert basic amino acids into cinnamic acid. Subsequently, cinnamic acid act as a precursor for most common phenolic compounds in plants [4]. Which may leads the increment of phenol compound after rejuvenation under this experiment. The cultivars were not varied statistically for total chlorophyll content (Fig .2) before and after rejuvenation. However, before rejuvenation it was recorded higher in cv. Himsagar (1.08 mg/g) followed by Fazli (1.05 mg/g). In contrast, Fazli was recorded higher chlorophyll content (0.96 mg/g) followed by Mallika (0.89 mg/g) (Table 1). It was found that the chlorophyll content was decreased after the rejuvenation and it was supported by [18].

Carbohydrates are the important components of storage and structural materials in plants. It was revealed (Table 2) that the total carbohydrate was not significantly varied before and after the rejuvenation practice. However, it was highest with cv. Himsagar before (0.80%) and after (0.74%) the rejuvenation. The carbohydrate content is decreased after rejuvenation on all the cultivars. Carbohydrate contents was decreased after rejuvenation by utilizing the carbohydrate reserves for bud emergence, production of leaves, shoots and other metabolic activities of plants. [8] reported that dormant pruning decreased starch and soluble sugars content in branches of apple trees. The high production of new shoots after pruning would be expected to decrease the reserves of nutrients, particularly carbohydrates, stored in the different parts of the tree, which are utilized for the production of bud formation [11]. The differences of carbohydrate content may be due to the genetical difference among the cultivars.

Total amino acids showed varied result among the cultivars studied under the experiment. After rejuvenation practice the total amino acids were not significantly different among the cultivars. The pooled data shows that the maximum amount of total amino acid was recorded with cv. Amrapali (1.31%) before the rejuvenation. However, after pruning it was recorded higher on cv. Himsagar (2.0 %). The increment of amino acid after rejuvenation may be due to inefficient utilization of amino acids in protein synthesis or due to proteolysis as suggested by [3]. The increment of amino acid content after pruning as due to the plant under gone stress following rejuvenation. The fact was stated by [21]. Increment in amino acids in stress may also due to the degradation of proteins [12]. The differences of amino acid may be due to the genetic differences among the cultivars.

Negligible difference for peroxidase activity was observed for year wise and pooled data before and after the rejuvenation practices. Maximum amount of peroxidase activity (0.37min/g) was recorded in cv. Himsagar (Table 3) before and after the rejuvenation and it was minimum (0.30 min/g) before and after rejuvenation for cv. Mallika.

The changes in PPO activity due to rejuvenation. The maximum activity of catechol oxidase (0.72) and laccase (0.62) were recorded (Fig.3) in Langra, whereas, minimum quantity catechol oxidase (0.60) and laccase (0.55) was recorded in Mallika and Amrapali before rejuvenation, respectively. The maximum activity of catechol oxidase (0.82) and laccase (0.72) were recorded in Langra, whereas, minimum quantity catechol oxidase (0.61) and laccase (0.64) was recorded in Mallika and Amrapali, after rejuvenation of mango plants respectively (Table 3). Plants after performing the rejuvenation process may developed a self-defensive mechanism by the increasing activity of peroxidase and polyphenol oxidase enzymes as studied by [3] and [19].

It was concluded that the rejuvenation effect on the physiological and biochemical status of different mango cultivars. Total phenol content was significantly different before and after the rejuvenation practices. It is also noticed that the phenol content was comparatively higher before the rejuvenation than after rejuvenation practices. The total carbohydrate was not significantly varied before and after the rejuvenation practice. However, it was highest with cv. Himsagar before and after the rejuvenation. Total amino acids showed varied result among the cultivars studied under the experiment. After rejuvenation practice the total amino acids were not significantly different among the cultivars. The maximum amount of total amino acid was recorded with cv. Amrapali before the rejuvenation. Maximum amount of peroxidase activity was recorded in cv. Himsagar before and after the rejuvenation and it was minimum before and after rejuvenation for cv. Mallika. The maximum activity of catechol oxidase and laccase were recorded in Langra, and minimum quantity catechol oxidase and laccase was recorded in Mallika and Amrapali before rejuvenation, respectively.

Future scope of the study

There is very meagre information on scientific literature regarding the biochemical changes of different mango cultivars on before and after rejuvenation. This study may helpful for conduct the deep research on biochemical changes in different mango cultivars in this sub Himalayan *Terai* region of West Bengal.

Conflict of interest

The authors do not have any conflict of interest

Acknowledgment

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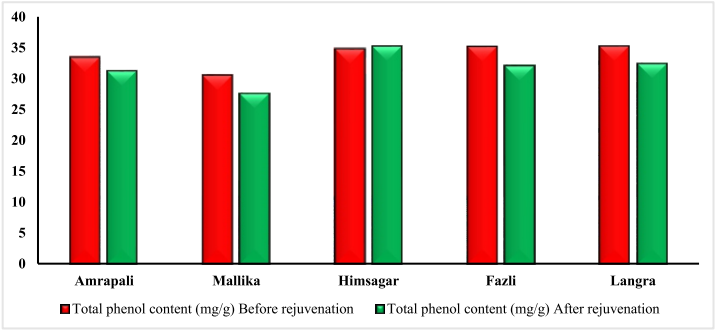

Fig .1. Impact of rejuvenation on total phenol content (mg/g) of different mango cultivars

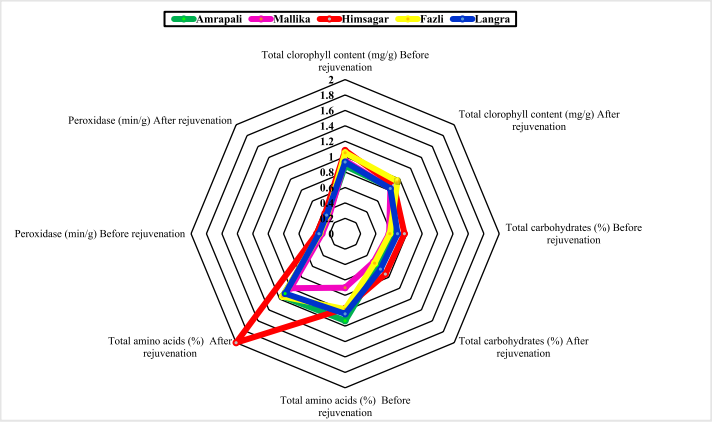

Fig .2. Impact of rejuvenation on total chlorophyll content (mg/g), total carbohydrates (%), total amino acids (%) and Peroxidase (min/g) of different mango cultivars

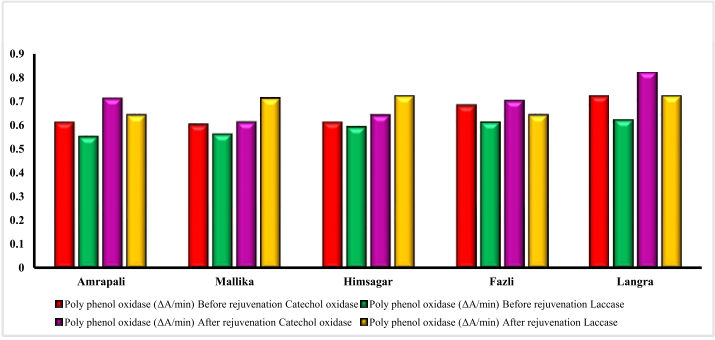

Fig 3. Impact of rejuvenation on Poly phenol oxidase (ΔA/min) of different mango cultivars

Table 1: Impact of rejuvenation on Total phenol content and Total chlorophyll content of different mango cultivars

	Total phenol content (mg/g)						Total chlorophyll content (mg/g)					
	Before rejuvenation			After rejuvenation			Before rejuvenation			After rejuvenation		
Treatments	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled
T1-Amrapali	31.25bc	35.50a	33.38ab	29.00a	33.25ab	31.13b	0.82a	0.94a	0.88a	0.78a	0.89a	0.84a
T2-Mallika	31.00c	30.00a	30.50b	29.75a	25.25c	27.50ab	1.16a	0.73a	0.95a	0.99a	0.67a	0.83a
T3-Himsagar	35.25b	34.25a	34.75a	35.00a	35.25a	35.13a	1.00a	1.16a	1.08a	0.81a	0.97a	0.89a
T4-Fazli	39.50a	30.75a	35.13a	36.00a	27.88bc	31.94ab	1.11a	0.99a	1.05a	0.98a	0.93a	0.96a
T5-Langra	35.00bc	35.38a	35.19a	31.00a	33.63ab	32.32ab	0.98a	0.87a	0.93a	0.95a	0.70a	0.83a
S.E m. (±)	1.36	1.85	1.06	2.63	2.14	2.08	0.16	0.21	0.11	0.12	0.15	0.06
L.S.D(P≤0.05)	4.20	5.72	3.27	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means with same letter are not significantly differs with each other

Table 2: Impact of rejuvenation on Total carbohydrates and Total amino acids of different mango cultivars

	Total carbohydrates (%)						Total amino acids (%)					
	Before rejuvenation			After rejuvenation			Before rejuvenation			After rejuvenation		
Treatments	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled
T1-Amrapali	0.62a	0.64a	0.63a	0.61a	0.59a	0.60a	1.09a	1.17a	1.13a	1.11a	1.18a	1.15a
T2-Mallika	0.73a	0.78a	0.76a	0.57a	0.47a	0.52a	0.71a	0.68b	0.70b	1.13a	0.86a	1.00a
T3-Himsagar	0.85a	0.75a	0.80a	0.77a	0.71a	0.74a	0.96a	0.98a	0.97a	1.39a	1.30a	2.0a
T4-Fazli	0.59a	0.66a	0.63a	0.58a	0.49a	0.54a	0.98a	0.97a	0.98a	1.12a	1.17a	1.15a
T5-Langra	0.61a	0.65a	0.63a	0.68a	0.61a	0.65a	1.02a	1.05a	1.04a	1.24a	0.95a	1.10a
S.E m. (±)	0.09	0.09	0.08	0.10	0.11	0.10	0.13	0.08	0.07	0.20	1.03	0.56
L.S.D(P≤0.05)	NS	NS	NS	NS	NS	NS	NS	0.24	1.71	NS	NS	NS

Means with same letter are not significantly differs with each other

Table 3: Impact of rejuvenation on Peroxidase and Poly phenol oxidase of different mango cultivars

	Peroxidase (min/g)						Poly phenol oxidase (ΔA/min)											
	Before rejuvenation			After rejuvenation			Before rejuvenation						After rejuvenation					
Treatments	2019-20	2020-21	Pooled	2019-20	2020-21	Pooled	2019-20		2020-21		Pooled		2019-20		2020-21		Pooled	
							Cat.	Lac.	Cat.	Lac.	Cat.	Lac.	Cat.	Lac.	Cat.	Lac.	Cat.	Lac.
T1-Amrapali	0.32a	0.32a	0.32a	0.31a	0.32a	0.32a	0.48a	0.49 a	0.73a	0.61a	0.61a	0.55a	0.69a	0.62a	0.73a	0.66a	0.71a	0.64a
T2-Mallika	0.30a	0.30a	0.30a	0.40a	0.37a	0.30a	0.52a	0.48a	0.68a	0.64a	0.60a	0.56a	0.54a	0.73a	0.68a	0.68a	0.61a	0.71a
T3-Himsagar	0.38a	0.35a	0.37a	0.34a	0.34a	0.37a	0.50a	0.47a	0.71a	0.70a	0.61a	0.59a	0.56a	0.59a	0.71a	0.84a	0.64a	0.72a
T4-Fazli	0.33a	0.33a	0.33a	0.36a	0.34a	0.33a	0.61a	0.40a	0.74a	0.57a	0.68a	0.61a	0.66a	0.66a	0.74a	0.62a	0.70a	0.64a
T5-Langra	0.35a	0.32a	0.34a	0.34a	0.34a	0.34a	0.57a	0.53a	0.86a	0.70a	0.72a	0.62a	0.78a	0.66a	0.76a	0.77a	0.82a	0.72a
S.E m. (±)	0.05	0.04	0.04	0.06	0.05	0.04	0.05	0.05	0.07	0.06	0.03	0.04	0.08	0.02	0.05	0.07	0.04	0.05
L.S.D(P≤0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means with same letter are not significantly differs with each other
Cat.: - Catechol oxidase; Lac.: - Laccase

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