Polyphenol oxidase activity in mango (*Mangifera indica* L.) in relation to flowering behaviour and the malformation incidence

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Polyphenol oxidase activity in mango (*Mangifera indica* L.) in relation to flowering behaviour and the malformation incidence.

Abstract — **Introduction**. Biennial bearing and malformation are the two present problems of mango which are threatening the very existence of a global mango industry in India. Many factors are closely associated with these problems but, presently, those really responsible are not known exactly. Polyphenol oxidase (PPO) plays vital roles in higher plants and, hence, its activity was measured in swollen buds at panicle initiation in regular and biennial bearing mango cultivars to establish a relation between the PPO activity and the flowering behaviour or the malformation incidence. **Materials and methods**. Six regular (Sensation, Eldon, Tommy Atkins, Amrapali, Neelum and Bangalora) and six biennial bearing cultivars (Langra, Chausa, Edward, St. Alexandrina, Extrema and Bombay Green) were studied. PPO activity was measured in fully swollen buds by preparing a crude enzyme extract at 4 °C. Catecholase activity was measured by using 4-methyl catechol (4-MC) as the substrate, while 4-methyl phenol (p-cresol) was used for cresolase. **Results and discussion**. Both catecholase and cresolase activities were higher in the swollen buds in the year 2000 than in 1999. Regular bearers exhibited low PPO activity and showed the higher incidence of malformation as compared to the biennial bearing cultivars.Whatever their type of production, cultivars exhibited significant variations in PPO activities and malformation incidence. **Conclusion**. A strong negative correlation between PPO activity and number of panicles, and PPO activity and malformation incidence, suggests that the PPO activity is inversely related to both flowering and malformation incidence in mango.

India / *Mangifera indica* / production / enzymic activity / catechol oxidase / flowering / malformations / fruits

Activité de la polyphénol oxydase chez la mangue (*Mangifera indica* L.) en relation avec la floraison et l'observation de malformation.

Résumé — **Introduction**. La production bisannuelle et la malformation du fruit sont les deux problèmes actuels qui menacent l'existence même d'une industrie globale de la mangue en Inde. Beaucoup de facteurs ont été étroitement associés à ces problèmes mais, à ce jour, ceux qui en sont réellement responsables ne sont pas exactement connus. La polyphénol oxydase (PPO) joue un rôle essentiel chez les plantes supérieures et, par conséquent, son activité a été mesurée dans des bourgeons gonflés au stade de l'initiation florale, chez des cultivars de manguiers à productions régulières ou bisannuelles, afin d'établir une relation entre l'activité de la PPO et la floraison ou l'observation de malformations. **Matériel et méthodes**. Six cultivars à production régulière (Sensation, Eldon, Tommy Atkins, Amrapali, Neelum et Bangalora) et six autres à production bisannuelles (Langra, Chausa, Edward, St. Alexandrina, Extrema et Bombay Green) ont été étudiés. L'activité de la PPO a été mesurée dans les bourgeons entièrement gonflés en préparant un extrait brut d'enzymes à 4 °C. L'activité de la catécholase a été mesurée en employant le méthyle-4 catéchol (4-MC) comme substrat tandis que le méthyl-4 phénol (p-crésol) a été employé pour étudier la crésolase. **Résultats et discussion**. Les activités de la catécholase et de la crésolase ont été plus élevées en 2000 qu'en 1999. Les cultivars à production régulière ont montré une basse activité de la PPO et davantage de malformations que les cultivars produisant tous les deux ans. Quel que soit leur type de production, les cultivars ont montré des variations significatives d'activités de la PPO et de taux de malformation des fruits. **Conclusion**. Une forte corrélation négative entre l'activité de la PPO et le nombre d'inflorescences, et entre cette même activité et le taux de malformations suggère que l'activité de la PPO est inversement liée à la floraison et aux anomalies de formation du fruit chez le manguier.

Inde / *Mangifera indica* / production / fréquence de récolte / activité enzymatique / catéchol oxydase / floraison / malformation / fruits

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1. Introduction

Mango, the choicest fruit of India, occupies a pre-eminent place amongst fruit crops and is acknowledged as "the king of fruits" in this country. There are about 1600 cultivars of mango in the world, of which about 1200 exist in India [1]. However, only a few cultivars have attained commercial status in the world mango industry.

There are many inherent problems associated with the mango industry in the world, but the problem of biennial bearing is one of the major problems as it renders mango cultivation less remunerative to the growers and is thus the major bottleneck in the expansion of the mango industry. Several aspects like climatological factors [2], age and size of the shoot [2–4], carbon:nitrogen ratio [5, 6], and hormonal imbalance [6–8] may be closely associated with biennial bearing, but, at the present time, no definite knowledge could explain which factors are responsible for biennial bearing in mango [9]. Polyphenol oxidase (PPO), a widely distributed enzyme in plants, has been studied thoroughly to assess its role in higher plants. Mayer and Harel [10] reported its association with *in vivo* synthesis and accumulation of phenolic compounds. Further, polyphenols have been reported to be associated with the formation of IAA – an auxin [11] and auxins are reported to be closely associated with vegetative growth and subsequent flowering in mango [6–8], although Briggs and Ray [12] and Tomaszewski and Thimman [13] have shown their doubts about this role of PPO.

Malformation, a disorder of international significance, affects with variable intensity, different mango cultivars (both regular and biennial bearers) in North India and in other parts of the world [14–16] and its incidence has been inversely related to PPO activity [16]. Thus, PPO activity (catecholase and cresolase) may have some relation to flowering, and studies were therefore conducted to establish a relation between PPO activity and flowering behaviour, PPO activity and malformation incidence, and flowering behaviour and malformation incidence in mango.

2. Materials and methods

Five uniformly maintained full bearing plants (14–15 year old) were selected for the studies. Each of them were regular ('Sensation', 'Eldon', 'Tommy Atkins', 'Amrapali', 'Neelum' and 'Bangalora') or biennial bearing ('Langra', 'Chausa', 'Edward', 'St. Alexandrina', 'Extrema' and 'Bombay Green'), with a known history of regularity / bienniality. In the years 1999 and 2000, fully swollen buds at the beginning of panicle initiation (March) were excised and used for measuring polyphenol oxidase (both catecholase and cresolase) activity. Enzyme assay was done in 24 samples per plant per variety. The total number of panicles healthy and malformed panicles in each variety were counted during March and averaged for calculating malformation incidence. The data on PPO activity of both the years were averaged, pooled and analysed by using simple split plot design [17]. Correlations between PPO activity and number of panicles (flowering behaviour), PPO activity and malformation incidence were calculated.

2.1. Preparation of crude enzyme extract

The crude enzyme extract was prepared at 4 ºC as per the procedure of Sharma et al. [16]. The swollen buds were chopped and mixed before preparing enzyme extract. One g of chopped material was homogenised with 5 mL of 100 mM of phosphate buffer (pH 7.3) containing 10 mM of sodium ascorbate in a blender for 15 s, filtered through four layers of gauze and centrifuged at 3,000 *g* for 30 min. The precipitate was re-extracted for 15 min with 5 mL of 1.5% Triton-X-100, prepared in 100 mM phosphate buffer (pH 7.3). The final volume of the extract was made up to 25 mL with phosphate buffer (pH 7.3). The filtrate was then centrifuged at 15,000 *g* for 1 h. An ammonium sulphate fractionation was carried out, and the fraction precipitating between 45% and 95% saturation was collected and re-dissolved. After dialysis, this solution was used as an enzyme source.

2.2. Enzyme assay

Both catecholase and cresolase activities were measured spectrophotometrically at 400 nm as per the procedure of Sharma et al. [16].

Catecholase activity was measured by using 30 mM of 4-methyl catechol (4MC) as substrate, made in 10 mM of sodium acetate buffer (pH 4.5). To 1 mL crude enzyme extract, 3 mL of 100 mM phosphate buffer (pH 7.3) was added. To this mixture, 1 mL of substrate was added at zero time and the change in absorbance at 400 nm was recorded in a CL-1200 spectrophotometer.

The cresolase activity was also measured similarly, except that 4-methyl phenol (p-cresol) was used as substrate made in 10 mM of phosphate buffer (pH 7.0). The enzyme activity was represented as change in absorbance at 400 nm per g of tissue weight per min ($\Delta A_{400} \times g^{-1} \times min^{-1}$).

3. Results

3.1. Polyphenol oxidase activity

Polyphenol oxidase (catecholase and cresolase) activity varied widely in different cultivars and both the seasons. Catecholase and cresolase activities were higher both in regular and biennial bearers during the year 2000 than in 1999. However, the rise in activities was comparatively higher in biennial bearers than in regular bearers (*table I*). In general, the regular bearing cultivars exhibited low PPO activity (0.657) as

Table I.
Polyphenol oxidase activity and malformation incidence in regular and biennial bearing mango cultivars in India.

Mango cultivar	Polyphenol oxidase activity (change in absorbance at 400 nm × g^{-1} × min^{-1})						Mean for PPO activity	No. of panicles emerged			Malformation incidence (%)		
	Catecholase			Cresolase									
	1999	2000	Mean	1999	2000	Mean		1999	2000	Mean	1999	2000	Mean
Regular bearers													
Eldon	0.752	0.926	0.839	0.462	0.624	0.543	0.691	152.8	130.6	141.7	43.90	41.30	42.60
Tommy Atkins	0.782	0.952	0.867	0.491	0.641	0.566	0.717	142.6	126.3	134.5	41.60	40.80	41.20
Amrapali	0.660	0.820	0.740	0.364	0.520	0.442	0.591	212.4	189.2	200.8	53.75	51.90	52.83
Sensation	0.735	0.895	0.815	0.440	0.596	0.518	0.667	166.5	155.2	160.9	44.10	42.60	43.35
Neelum	0.698	0.862	0.780	0.400	0.556	0.478	0.629	196.3	179.5	187.9	52.90	50.60	51.75
Bangalora	0.717	0.879	0.798	0.416	0.568	0.492	0.645	173.2	160.3	166.8	46.30	43.80	45.05
Mean	0.724	0.889	0.807	0.428	0.585	0.507	0.657	174.0	156.9	165.5	47.09	45.17	46.13
Biennial bearers													
Extrema	0.951	1.133	1.042	0.840	0.980	0.910	0.976	118.3	11.9	65.1	36.50	34.00	35.25
Bombay Green	0.920	1.102	1.011	0.817	0.961	0.889	0.950	126.2	12.0	69.1	37.20	34.60	35.90
Langra	0.897	1.075	0.986	0.726	0.876	0.801	0.894	133.2	18.5	75.9	39.60	37.80	38.70
Chausa	0.908	1.088	0.998	0.801	0.943	0.872	0.935	130.5	12.5	71.5	39.00	37.00	38.00
St. Alexandrina	0.996	1.166	1.081	0.923	1.073	0.998	1.040	112.6	11.9	62.3	30.85	29.55	30.20
Edward	0.981	1.145	1.063	0.901	1.043	0.972	1.018	115.1	12.1	63.6	33.12	31.84	32.48
Mean	0.942	1.118	1.030	0.835	0.979	0.907	0.969	122.7	13.2	67.9	36.10	34.13	35.12
Significance	Cultivar = 0.004 Year = 0.005 Cultivar × year = 0.006			Cultivar = 0.003 Year = 0.003 Cultivar × year = 0.005				Cultivar = 4.8 Year = 9.3 Cultivar × year = 14.1			Cultivar = 2.76 Year = 4.12 Cultivar × year = 6.90		

compared to the biennial bearers (0.969). Even within regular or biennial bearers, a wide variability was observed both in catecholase and cresolase activity. Among the regular bearers, 'Tommy Atkins' exhibited the highest (0.717) and 'Amrapali' the lowest PPO activity (0.591). In biennial bearers, 'St. Alexandrina' exhibited the highest PPO activity (1.040) and 'Langra' the lowest (0.894) (*table I*). Irrespective of cultivar, the catecholase activity (0.807 and 1.030) was at all times higher than the cresolase activity (0.507 and 0.907) in the regular and biennial bearers, respectively. Further, the trend of cresolase activity in the regular and biennial bearers, was similar to catecholase activity, though it was quite lower than catecholase activity (*table I*).

3.2. Panicle emergence

Regular and biennial bearing cultivars produced varying numbers of panicles in both the years. The regular bearers produced as many as 165.5 panicles per plant as compared to only 67.9 by the biennial bearers. Irrespective of cultivar, regular bearers produced 174.0 panicles per plant in 1999 and 156.9 panicles in the year 2000. However, the biennial bearers produced a good amount of panicles in 1999 (122.7) but only 13.2 panicles in the year 2000. Among the regular bearers, 'Amrapali' produced the maximum number of panicles (200.8) and 'Tommy Atkins' the minimum (134.5). Similarly, among the biennial bearers, 'Langra' produced the highest number of panicles (75.9) and 'St. Alexandrina' the lowest (62.3) (*table I*).

3.3. Malformation incidence

The incidence of malformation was observed to be non-significant between the years but significant between the cultivars. In general, the malformation incidence in the regular bearers was significantly higher (46.13%) than in the biennial bearers (35.12%). Further, all the regular bearers were more infested with malformation than the biennial bearers (*table I*). Among the regular bearers, 'Amrapali' had the maximum incidence of malformation (52.83%) and 'Tommy Atkins' had the minimum (41.20%). Similarly, in biennial bearers, 'Langra' was worst affected by malformation (38.70%) and 'St. Alexandrina' had the least incidence (30.20%) (*table I*).

3.4. Correlation

An inverse relation ($r = –0.890$) was observed between PPO (catecholase and cresolase) activity and the number of panicles formed. Similarly, the correlation between PPO activity and malformation was also strongly negative ($r = –0.913$).

4. Discussion

There exists wide variation among different mango cultivars in respect to catecholase and cresolase activity (*table I*). This variation in enzyme activity in different cultivars may be attributed to genetic differences [16]. The regular bearing cultivars exhibited low PPO activity as compared to the biennial bearers. PPO is responsible for *in vivo* synthesis of phenolic compounds, which are secondary metabolites [10, 18]. Organic materials in terms of manures and fertilisers are used every year for the production of new vegetative growth, subsequent flowering and fruiting process in regular bearers, whereas these are kept reserved in the 'off' year in the alternate bearers [8]. Thus, low catecholase and cresolase (PPO) activity in regular bearers may be due to the dilution of polyphenols in the 'on' year and *vice versa*. Moreover, PPO is involved in the formation of auxin (IAA) by acting on tryptophan [11] and high concentration of auxin favours vegetative growth, which may inhibit the flowering and fruiting process in mango [6–8]. Thus, higher enzyme activity in alternate bearers favours vegetative growth and inhibits flowering during the 'off' year in mango. Further, the catecholase activity was at all times higher than that of cresolase activity, both in regular and biennial bearing cultivars. Lower cresolase activity may probably be due to its lag period, greater instability and rapid loss during its extraction [16, 19].

A wide variability was observed in the emergence of panicles in regular and biennial bearers in both years (*table I*). The regular bearers flowered in both years, whereas the flowering process was reduced drastically in the biennial bearers during 2000. The year 1999 being the 'on' year, alternate bearers produced panicles but induced very few panicles in the year 2000, being the 'off' year for them. Among the regular bearers, 'Amrapali' produced the highest number of panicles (200.8) and 'Tommy Atkins' the lowest (134.5). Similarly, among the alternate bearers, 'Langra' produced the maximum (75.9) and 'St. Alexandrina' the minimum number of panicles (62.3). This variable response of the regular and biennial bearers to the flowering may be due to the genetical and inherent factors associated with different cultivars responsible for fruiting in mango [8]. Further, flowering behaviour can also be correlated with catecholase and cresolase activity. The enzyme activity in the regular bearers had been low and these produced a higher number of panicles. In contrast, the biennial bearers had exhibited the higher PPO activity and these produced a lower number of the panicles (*table I*). Among the regular bearers, 'Amrapali' had the lowest catecholase and cresolase activity and produced the maximum number of panicles (200.8) and 'Tommy Atkins', which had the highest enzyme activities, produced the lowest number of panicles (134.5). Further, the strongly negative correlation (r = –0.890) between the enzyme activity and the number of panicules indicates the inverse relation with the regularity in bearing, as low activity had favoured the regularity and the high activity the bienniality in mango.

The intensity of malformation also differed greatly among different mango cultivars. In general, the incidence of malformation was higher in regular bearing cultivars (46.13%) than in the biennial bearing ones (35.12%). Higher malformation incidence in regular bearing cutivars may be due to low PPO activity, which might have resulted in poor synthesis of phenolic compounds [16]. These compounds are generally responsible for the defence mechanism in plants [10, 18]. Among the regular bearers, 'Amrapali' had the highest incidence of malformation (52.83%) and had exhibited the lowest PPO activity (0.591) and 'Tommy Atkins' had the least incidence of malformation (41.20%) and had exhibited the highest activity (0.717). Similarly, among the regular bearers, 'Langra' was the worst affected by malformation (38.70%) and 'St. Alexandrina' the least (*table I*). This variation in malformation incidence among the cultivars may be due to genetic variability and inherent factors associated with the susceptibility to or resistance against malformation [16]. Further, the correlation between PPO activity and malformation incidence is strongly negative, showing an inverse relation between PPO activity and malformation incidence, i.e., higher the PPO activity, the lower the malformation, and lower the PPO activity, higher the malformation incidence.

5. Conclusions

Low PPO activity favours flowering (panicle development) and incidence of malformation, whereas, high PPO favours bienniality and disfavours malformation incidence in mango. Further, the regular bearers are more prone to the development of malformation than the biennial bearers.

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Actividad de la polifenol oxidasa en el mango (*Mangifera indica* L.) con relación a la floración y la observación de malformación.

Resumen — **Introducción**. La producción bianual y la malformación del fruto son los dos problemas actuales que amenazan la existencia de una industria global del mango en India. Muchos factores han sido estrechamente asociados a estos problemas pero, hasta ahora, no se conocen con exactitud los que son realmente responsables. La polifenol oxidasa (PFO) desempeña un papel esencial en las plantas superiores. Por consiguiente, se midió su actividad en yemas hinchadas en la fase de la iniciación floral, en cultivares de mangos con producciones anuales o bianuales, para establecer una relación entre la actividad de la PFO y la floración o la observación de malformaciones. **Material y métodos**. Seis cultivares de producción regular (Sensation, Eldon, Tommy Atkins, Amrapali, Neelum y Bangalora) y otros seis de producción bianual (Langra, Chausa, Edward, St. Alexandrina, Extrema y Bombay Green) fueron estudiados. Se midió la actividad de la PFO en las yemas completamente hinchadas preparando un extracto bruto de enzimas a 4 °C. Se midió la actividad de la catecolasa empleando el metilo-4 catecol (4-MC) como substrato mientras que el metil-4 fenol (p-cresol) se empleó para estudiar la cresolasa. **Resultados y discusión**. Las actividades de la catecolasa y de la cresolasa fueron más altas en 2000 que en 1999. Los cultivares de producción regular han mostrado una baja actividad de la PFO y más malformaciones que los cultivares bianuales. Sea cual fuere su tipo de producción, los cultivares mostraron variaciones significativas de las actividades de la PFO y de la tasa de malformación de los frutos. **Conclusión**. Una fuerte correlación negativa entre la actividad de la PFO y el número de inflorescencias y entre esta misma actividad y la tasa de malformación sugiere que la actividad de la PFO está inversamente relacionada con la floración y las anomalías en la formación del fruto en el mango.

India / *Mangifera indica* / producción / frecuencia de las cosechas / actividad enzimática / catecol oxidasa / floración / malformación / frutas