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Endogenous Hormones and Phenols in Rootstock Seedlings of Mango Cultivars and their Relationship with Seedling Vigour

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Summary

Studies on morpho-physiological characters, contents of total phenols and endogenous hormones in the seedlings of seven polyembryonic mango cultivars, 'Muvandan', 'Vellaikolamban', 'Chandrakiran', 'Kurukan', 'Bappakai', 'Mylepelian' and 'Olour' and monoembryonic cultivar, 'Alphonso' raised under pot culture conditions monitored at 30, 60 and 120 days of growth from transplanting revealed that the seedling vigour of polyembryonic cvs 'Muvandan' and 'Bappakai' and of the monoembryonic cv. 'Alphonso' was high. The cv. 'Vellaikolamban' was least vigorous. The leaf area at 60 days of seedling growth was low in less vigorous cvs 'Kurukan' and 'Vellaikolamban' and high in 'Muvandan'. The leaf water potential was less negative in less vigorous cvs 'Vellaikolamban', 'Kurukan' and 'Chandrakiran'. The total phenol and ABA in the leaves were high in less vigour cvs 'Vellaikolamban' and 'Kurukan' at all seedling growth stages. The leaves of vigorous cvs 'Bappakai' and 'Muvandan' had high cytokinins, *t*-zeatin riboside and dihydrozeatin zeatin riboside levels at 30, 60 and 120 days and IAA level at 30 and 60 days. Plant height and stem girth were negatively correlated with leaf total phenol and ABA contents. Plant height was positively correlated with leaf water potential and cytokinins (*t*-zeatin riboside and dihydrozeatin zeatin riboside) content. Thus a higher level of growth inhibitor, ABA and phenols concurrently with lower levels of growth promoters imparted growth restriction of seedlings. It is suggested that the selection of size controlling rootstocks at nursery stage can be made by assessing the leaf water potential, total phenols and/or ABA.

Zusammenfassung

Endogene Hormone und Phenole in Sämlingsunterlagen von Mango und ihre Auswirkungen auf die Wüchsigkeit der Sämlinge. Untersuchungen zu morphologisch-physiologischen Eigenschaften, dem Gesamtgehalt an Phenolen und endogenen Hormonen in den Sämlingen von sieben mehrkeimigen Mangosorten, 'Muvandan', 'Vellaikolamban', 'Chandrakiran', 'Kurukan', 'Bappakai', 'Mylepelian' und 'Olour', sowie der einkeimigen Sorte 'Alphonso', die in Töpfen kultiviert und 30, 60 und 120 Tage nach der Umsetzung bonitiert wurden, zeigten, dass die Wüchsigkeit der mehrkeimigen Sorten 'Muvandan' und 'Bappakai' sowie der einkeimigen Sorte 'Alphonso' groß war.

Die Sorte 'Vellaikolamban' war am schwachwüchsigsten. 60 Tage nach dem Verpflanzen waren die Blattflächen der schwächerwüchsigen Sorten 'Kurukan' und 'Vellaikolamban' am kleinsten und die von 'Muvandan' am größten. Das Wasserpotential der Blätter war bei den schwächerwüchsigen 'Vellaikolamban', 'Kurukan' and 'Chandrakiran' weniger stark negativ. Der Gehalt an Gesamtphenolen und ABA in den Blättern war im gesamten Versuchszeitraum bei den schwächerwüchsigen Sorten 'Vellaikolamban' und 'Kurukan' am höchsten. Die Blätter der wüchsigen Sorten 'Bappakai' und 'Muvandan' hatten nach 30, 60 und 120 Tagen hohe Cytokinin-, *t*-Zeatinribosid- und Dihydrozeatin Zeatinribosid-Werte und nach 30 und 60 Tagen hohe IAA-Werte. Pflanzenhöhe und Stammumfang waren negativ korreliert mit dem Gesamtphenol- und ABA-Gehalt der Blätter. Pflanzenhöhe und Wasserpotential der Blätter sowie Cyokinin- (*t*-Zeatinribosid- und Dihydrozeatin Zeatinribosid-) Gehalt sind positiv korreliert. Folglich führt ein höherer Gehalt an Wuchshemmstoffen, ABA und Phenolen in Verbindung mit einem geringeren Gehalt an Wachstumspromotoren zu Wachstumsbeschränkungen der Sämlinge. Es wird vorgeschlagen, die Selektion auf Schwachwüchsigkeit im Baumschulstadium durch Bestimmung des Wasserpotentials der Blätter, der Gesamtphenole und/oder ABA durchzuführen.

Key words. mango – cytokinins– ABA – IAA – phenols – vigour – rootstock

Introduction

High-density planting has received considerable emphasis for enhancing orchard efficiency and productivity in fruit crops. The importance of dwarf trees in high-density orcharding, in general, and of rootstocks in the regulation of vigour of trees, in particular, has received attention in fruit trees such as apple. Mango is an important commercially grown tropical fruit crop that shows considerable variation in growth habits, vigour and flowering. The rootstocks have been shown to considerably influence scion growth in mango

(MURTI et al. 2000). The physiological mechanism by which rootstocks impede the growth and development of grafted trees is not well understood. In a study made on fully grown seedling trees, we reported that the dwarfing potential of polyembryonic mango cultivars used as rootstocks is related to lower production of xylem sap, higher content of growth inhibitor, ABA and lower levels of growth promoters in xylem sap and leaves together with higher leaf phenol content (MURTI et al. 2000). Thus it was opined if similar trends in these parameters persist at seedling stage, the screening of rootstocks for the vigour could be done at nursery stage. Screening for vigour in mango at nursery stage was attempted earlier and these techniques were based on anatomical features (SINGH et al. 1986; MUKHERJEE and DAS 1976b), stomatal distribution (CHAKLADAR 1967), stomatal count (SRIVASTAVA et al. 1980), bark percentage (MUKHERJEE and DAS 1976a), leaf chlorophyll, total dry matter (PAL et al. 1981) and phenolics (BABU et al. 1985; KURIAN et al. 1994) at seedlings stage. The results were either inconclusive or inconsistent. As a follow-up of earlier study, we analyzed the levels of endogenous hormones, ABA, cytokinins and indole acetic acid (IAA) and total phenols in the leaves of seedlings of 7 polyembryonic mango cultivars and a monoembryonic cultivar, and related these with their vigour determining morphological attributes in order to arrive at some indices involved in vigour regulation with the ultimate objective of utilizing the same for early selection.

Materials and Methods

Experiments were conducted at the research farm of Indian Institute of Horticultural Research, Hessaraghatta during August–November, 2001. Seedlings of 7 polyembryonic mango cultivars, 'Bappakai', 'Chandrakiran', 'Kurukan', 'Muvandan', 'Mylepelian', 'Olour' and 'Vellaikolamban' and mono embryonic cultivar, 'Alphonso' were raised in sand mixed 3 x 5 soil beds. After 60 days of germination, the seedlings with healthy and uniform growth were transplanted to pots containing farmyard manure and garden soil (1:2). The experiment was conducted in 4 replicates under CRD design. The plants were irrigated daily with uniform volume of water.

Observations were taken on morphological characters, plant height, leaf number and stem girth at 30, 60 and 120 days after transplanting. The leaf area was measured at final sampling (120 days). In addition, leaf samples were also drawn for leaf water potential and for determining the contents of total phenols and endogenous hormones, abscisic acid (ABA), cytokinins, *t*-zeatin riboside and dihydrozeatin zeatin riboside (*t*-ZR and DHZR), and indole acetic acid (IAA). Gas exchange parameters were recorded at 60 days. For leaf water potential, the leaf discs of uniform size were cut and measurements were taken in Dew Point Microvolt Meter (Model HR-33T, Wescor, USA). The leaf water potential values were expressed as MPa. Gas exchange parameters and leaf area were recorded using portable photosynthesis system (Model 6200, LICOR, USA) and Leaf Area Meter (Model 3100, LICOR, USA), respectively. Total phenol content in leaves was determined by colorimetric procedure. The leaf sample (2 g) extracted in 80 % ethanol was boiled for 3 minutes and cooled further to room temperature. After filtering, the filtrate was extracted thrice with petroleum ether (b. p. 40–60 °C). The aqueous ethanolic extract was collected for 'Olour' development with Foiln-Ciocalteu reagent (BRAY and THORPE 1954) and the absorption was recorded at 660 nm after 1 h. The quantification of phenols was done using chlorogenic acid (Sigma make) as standard. The endogenous hormones, ABA (WEILER 1982), cytokinins, *t*-ZR and DHZR (BARTHE and STEWART 1985) and IAA (WEILER et al. 1981) in leaves were analyzed employing ELISA with laboratory raised polyclonal antibodies. Statistical analysis was performed according to the procedures of GOMEZ and GOMEZ (1984).

Results and Discussion

Morphological characters

Significant differences were observed in plant height, leaf number and stem girth at all the growth stages and in leaf area at 60 days among the cultivars (Table 1 and

Table 1. Plant height (cm), leaf number and stem girth (cm) in the seedlings of seven polyembryonic cultivars and a monoembryonic cultivar at different growth stages.

Cultivars	Days after transplanting								
	30 days			60 days			120 days		
	Plant height	Leaf no.	Stem girth	Plant height	Leaf no.	Stem Girth	Plant height	Leaf no.	Stem girth
Polyembryonic cultivars									
'Bappakai'	21.8	9.4	2.76	26.0	12.2	2.80	39.6	17.6	3.30
'Muvandan'	20.3	7.8	2.20	26.9	13.4	2.80	42.4	24.2	3.20
'Olour'	20.6	12.4	2.36	22.2	12.8	2.52	31.0	24.0	3.04
'Mylepelian'	20.1	8.8	2.16	27.6	12.8	2.80	33.6	17.2	2.80
'Chandrakiran'	18.1	4.8	1.70	20.5	4.6	2.04	23.0	9.4	2.20
'Kurukan'	13.8	5.8	1.74	18.2	9.8	1.80	25.1	13.2	1.80
'Vellaikolamban'	16.7	4.8	1.70	16.9	6.2	2.00	17.0	7.4	1.80
Monoembryonic cultivar									
'Alphonso'	27.2	6.4	2.36	29.0	7.2	2.52	37.1	12.4	2.75
CD 5 %	2.33	3.92	0.05	2.96	4.11	0.07	3.51	4.75	0.11
SEM	1.07	2.00	0.03	1.24	1.91	0.03	1.31	2.26	0.05

2). The plant height was higher in the seedlings of polyembryonic cultivars, 'Muvandan' and 'Bappakai' and lower in cvs 'Vellaikolamban', 'Kurukan' and 'Chandrakiran' consistently at all the stages. The leaf production was pronounced in cvs 'Muvandan', 'Olour', 'Bappakai' and 'Mylepelian' while it was low in cvs 'Vellaikolamban', 'Chandrakiran' and 'Kurukan'. The cvs 'Kurukan', 'Vellaikolamban' and 'Chandrakiran' consistently recorded lower stem girth (Table 1). At all the growth stages, the cv. 'Bappakai' had maximum stem girth followed by 'Muvandan' or 'Olour'. Stem girth exhibited significant positive correlation with the plant height (r=0.842 at 1%) and leaf number (r=0.850 at 1%). This suggested that the vigour potential of polyembryonic mango cultivars at different seedling growth stages differed considerably. While cv. 'Bappakai' and 'Muvandan' are highly vigorous, the cvs 'Vellaikolamban', 'Kurukan' and 'Chandrakiran' were less vigorous at seedling stage. Similar trends in vigour were observed earlier in the fully grown trees of these cultivars (MURTI et al. 2000). Leaf area was high in cvs 'Bappakai' and 'Muvandan' and low in cvs 'Vellaikolamban' and 'Kurukan' at 60 days (Table 2). The cv. 'Olour' inspite of showing large leaf number, showed lower leaf area due to smaller leaf size. KURIAN and IYER (1997) analyzed the leaf area in 24 mango cultivars, and reported that leaf area in vigorous cultivar is greater than that in dwarf trees. AGARWAL (1986) reported a high degree of positive correlation between leaf area and vigour of trifoliate orange. The similarity in the trends of leaf area in seedling and fully grown trees in mango cultivars reveals that this morphological attribute has a potential as an easy tool for vigour assessment at nursery stage.

Gas exchange parameters

The net photosynthesis (3.44–12.19 $\mu mol\, m^{-2}\, s^{-1}$), and stomata conductance (0.051–0.138 $\mu mol\, m^{-2}\, s^{-1}$) in the leaves showed considerable variation among seedlings of the cultivars at 60 days of growth. Net photosynthesis at this stage recorded did not reveal any definite trend with respect to vigour (Table 2). The stomatal conductance was high in less vigorous cvs 'Kurukan' and 'Vellaikolamban' and low in the more vigorous cv. 'Muvandan' (Table 2). Neither of the above gas change parameters showed any significant correlation with the vigour imparting morphological feature.

Leaf water potential (μ_w)

Distinct cultivar differences were observed in the leaf μ_w under saturated moisture condition at different stages of seedling growth. The dwarf cvs 'Vellaikolamban' and 'Kurukan' had less negative leaf μ_w, followed

Table 2. Leaf net photosynthesis rate ($\mu mol\, m^{-2}\, s^{-1}$) and stomata conductance ($\mu mol\, m^{-2}\, s^{-1}$) at 60 days and leaf area (cm^2) at 120 days after transplanting in the seedlings of seven polyembryonic cultivars and a monoembryonic cultivar.

Cultivars	Leaf area	Net photosynthesis rate	Stomata conductance
Polyembryonic cultivars			
'Bappakai'	12.19	0.059	52.46
'Muvandan'	7.39	0.065	49.83
'Olour'	9.29	0.081	33.13
'Mylepelian'	6.65	0.075	43.22
'Chandrakiran'	5.99	0.051	36.54
'Kurukan'	12.19	0.138	30.67
'Vellaikolamban'	7.21	0.087	23.71
Monoembryonic cultivar			
'Alphonso'	3.44	0.051	53.59
CD 5%	3.68	0.009	5.67
SEM	1.92	0.005	2.49

Table 3. Leaf water potential (–MPa) in the seedlings of seven polyembryonic cultivars and a monoembryonic cultivar at different growth stages.

Cultivars	Days after transplanting		
	30 days	60 days	120 days
Polyembryonic cultivars			
'Bappakai'	2.489	2.978	2.920
'Muvandan'	2.333	2.178	3.253
'Olour'	2.022	2.178	1.747
'Mylepelian'	2.267	2.333	2.427
'Chandrakiran'	1.822	1.933	1.440
'Kurukan'	2.156	1.822	1.320
'Vellaikolamban'	1.356	1.600	1.240
Monoembryonic cultivar			
'Alphonso'	2.822	2.356	2.427
CD 5%	0.487	0.582	0.614
SEM	0.233	0.265	0.312

by 'Chandrakiran' at 60 and 120 days of sampling (Table 3). It was more negative in the high vigour cvs 'Bappakai' and 'Muvandan'. It is of interest to note that leaf μ_w showed significant negative correlation with plant height (r=–0.821 at 1%). Not much literature is available on the plant water relations in relation to vigour regulation in perennial crops. Our results indicate a role for leaf μ_w in vigour regulation. The leaf μ_w defines the hydraulic conductivity of a plant from root to shoot for normal physiological and metabolic activities. The more negative μ_w in vigorous rootstock seedlings helps the seedlings in absorbing/translocating the available water from soil. This capacity for greater water absorption might be useful for the plants in maintaining a higher growth, as evident in vigorous mango seedlings. Further detailed studies are required on plant water relations in establishing the importance of water potential in vigour regulation in perennial crops.

Total phenols

At 30, 60 and 120 days after growth, the total phenol content in the leaves of mango seedlings ranged between 19.50–60.87, 20.69–64.42 and 9.46–59.10 mg g^{-1}, respectively. At all the stages, it was highest in less vigour cv. 'Vellaikolamban' followed by 'Kurukan' (Table 4). The phenols in high vigour cvs 'Bappakai', 'Muvandan' and 'Alphonso' were low. The total phenol content in leaves showed significant inverse relationship with the plant height (r=–0.958 at 1%) and stem girth (r=–0.857 at 1%) of cultivars. This shows that phenols play a vital role in the restriction of vigour of mango seedlings. Special phenols are known to exert an inhibitory effect on CD and CE processes as evident from the works of SWENSSON (1971). In our earlier studies also we witnessed a similar trend in total phenols content in the 20 year seedling trees of these mango cultivars (MURTI et al. 2000). Thus differential accumulation of phenols in the leaves as seen at seedling stage is also maintained in the fully grown trees. The vigour control exercised by rootstocks on scion could be attributed to the supply of phenols by them. Further studies on the individual phenols need to be taken up to see which of these is associated with the dwarfing habit of the rootstocks.

Endogenous hormones

At all the growth stages, the less vigorous cv. 'Vellaikolamban' followed by 'Kurukan' and 'Chandrakiran' recorded high ABA content in leaves (Table 5). The

Table 4. Total phenol content (mg g^{-1}) in the seedlings of seven polyembryonic cultivars and a monoembryonic cultivar at different growth stages.

Cultivars	Days after transplanting		
	30 days	60 days	120 days
Polyembryonic cultivar			
'Bappakai'	24.31	24.23	9.46
'Muvandan'	20.69	25.41	22.46
'Olour'	26.60	31.91	31.92
'Mylepelian'	32.51	25.41	14.78
'Chandrakiran'	36.64	43.14	39.60
'Kurukan'	46.69	54.37	50.24
'Vellaikolamban'	60.87	64.42	59.10
Monoembryonic cultivar			
'Alphonso'	19.50	20.69	19.50
CD 5%	4.32	3.94	4.96
SEM	2.20	1.95	2.41

Table 5. ABA and IAA content (ng g^{-1}) in the seedlings of seven polyembryonic cultivars and a monoembryonic cultivar at different growth stages.

Cultivars	Days after transplanting					
	30 days		60 days		120 days	
	ABA	IAA	ABA	IAA	ABA	IAA
Polyembryonic cultivar						
'Bappakai'	14.45	21.09	21.20	9.46	10.60	8.46
'Muvandan'	21.95	10.27	11.76	7.23	13.68	15.48
'Olour'	30.45	12.81	39.89	8.45	22.40	8.98
'Mylepelian'	18.90	9.38	26.35	6.59	20.81	8.67
'Chandrakiran'	37.90	7.62	49.02	8.42	26.94	11.09
'Kurukan'	47.70	7.08	57.35	5.73	28.97	7.90
'Vellaikolamban'	79.50	9.26	63.36	5.06	43.19	11.42
Monoembryonic cultivar						
'Alphonso'	16.60	24.82	10.70	15.14	11.40	12.90
CD 5%	7.52	2.94	9.11	2.58	5.69	3.41
SEM	3.84	1.55	4.35	1.24	3.04	1.63

Table 6. *t*-Zeatin riboside and dihydrozeatin zeatin riboside content (pg g^{-1}) in the seedlings of seven polyembryonic cultivars and a monoembryonic cultivar at different growth stages.

Cultivars	Days after transplanting					
	30 days		60 days		120 days	
	t-ZR	DHZR	t-ZR	DHZR	t-ZR	DHZR
Polyembryonic cultivar						
'Bappakai'	126.80	95.40	131.72	91.62	152.89	82.52
'Muvandan'	118.70	72.05	117.34	68.91	78.25	29.06
'Olour'	49.80	60.85	65.34	41.73	49.35	36.32
'Mylepelian'	81.10	43.90	67.73	28.47	50.00	30.09
'Chandrakiran'	39.30	37.80	46.28	23.19	34.67	29.05
'Kurukan'	35.55	30.20	39.48	24.40	28.47	24.96
'Vellaikolamban'	30.95	28.85	24.84	25.23	28.50	23.64
Monoembryonic cultivar						
'Alphonso'	138.80	159.00	180.14	134.43	119.14	106.25
CD 5%	10.22	11.03	13.25	8.64	9.84	8.12
SEM	5.17	5.41	6.08	4.11	4.31	4.51

ABA levels were low in the vigorous polyembryonic cvs 'Bappakai' and 'Muvandan'. Cytokinins, *t*-ZR and DHZR were higher in vigorous seedlings of 'Bappakai' and 'Muvandan' and lower in the less vigorous seedlings of cvs 'Vellaikolamban' and 'Kurukan', with *t*-ZR levels exhibiting greater variation than DHZR (Table 6). The leaf IAA broadly followed trends similar to that of cytokinins at 30 and 60 days of seedling growth (Table 5) but the differences in the IAA content amongst cultivars were small. Similar trends in these hormones in leaves and xylem sap were observed in the seedling trees of these polyembryonic cultivars (MURTI et al. 2000). Correlation analysis revealed that ABA is negatively related with the seedling plant height (r=–0.971 at 1%) and stem girth (r=–0.843 at 1%). While IAA did not exhibit any relationship with any of the morphological characters, the cytokinins, *t*-ZR (r=0.838 at 1%) and DHZR (r=0.721 at 5%) were positively correlated with the plant height. The higher levels of ABA in association with lower levels of cytokinins as recorded in low vigour cvs 'Vellaikolamban' and 'Kurukan' appear to play a vital role in vigour restriction of these seedlings. Shoot growth retardation with enhanced ABA levels was reported in apple (YADAVA and DAYTON 1972; JINDAL et al. 1974). YADAVA and LOCKARD (1977) reported higher levels of ABA in the leaves of dwarf clones of apple. We reported higher levels of ABA during active growth phase in the dwarf cultivar of mango as compared to vigorous one (MURTI and UPRETI 1998). KAMBOJ et al. (1999) observed a positive correlation between growth potential of the rootstock and cytokinin levels in xylem sap. SORCE et al. (2002) also recorded high ZR levels in the most invigorating rootstock of peach. JINDAL et al. (1974) witnessed that the shoot extracts from dwarf mutants of apple contained less auxin than those from normal plants and concluded that the levels of auxins may contribute to control the size of the plants. NODA et al. (2000) found positive correlation between the shoot tip IAA content and the shoot growth of lemon scion grafted on rootstock characterized by different invigorating properties. BUTA et al. (1989) found that the IAA levels in the leaves of vigorous apple trees were higher in early and late growing season as compared to those in dwarf one. These reports are in conformity with the results reported here.

In conclusion, the vigour expression of mango cultivars being used as rootstock at seedling stage is similar to that found in the fully grown trees. Leaf area, leaf water potential values and the levels of phenols and endogenous hormones, ABA and cytokinins in the leaves characterize the differences in vigour in seedlings. Higher levels of growth inhibitor, ABA and phenols and lower levels of growth promoters, cytokinins were associated with growth restriction of seedlings; an opposite trend in these favoured vigorous seedling growth. Whether the amount of phenols is the cause or effect of altered vigour is not clear from these studies. However, in an earlier investigation the same dwarfing rootstocks having higher phenols led to growth restriction in scion of cv. 'Alphonso' (MURTI et al. 2000). Based on these results it could be stated that the selection of genotype for vigour at nursery stage can be made by assessing the total phenols and the levels of hormones especially ABA. The role of leaf μ_w in vigour regulation needs a more detailed study.

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