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Propagule size affects yield and quality of Curcuma mangga Val. et Zijp.: An important medicinal spice

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ABSTRACT

Curcuma mangga is a medicinally important species grown in tropical Asian nations and is known to yield rhizomes that are source of curcumin and essential oil. This species has been valued in both traditional as well as modern medicines and has wide applications in cosmetic and pharmaceutical industries. However, the species has largely remained underutilized and its systematic cultivation could help in assuring continuous supply of uniform quality raw material to meet the industrial demands. During present investigation, effect of different size groups of seed rhizome was studied on yield and quality parameters, which revealed size dependent differences. Considering higher dry matter recovery (24.44%), oil yield (17.57 ml/m²) and curcumin content (0.46%), use of seed rhizomes of 20–25 g size could be recommended for producing raw material meant for aroma and pharmaceutical industries. On the other hand, if the final produce is meant for processing and value addition, use of seed rhizomes of 15–20 g size would be optimum as it would save the seed rhizome requirement without compromising with the yield. GC–MS analysis revealed β- Myrcene and Cyclofenchene as dominant constituents in essential oil of mother, primary and secondary rhizomes. Findings of present study would be helpful for large scale production of raw material required by flavor, food and pharmaceutical industries.

1. Introduction

The genus Curcuma with ca. 80 species is widely known for its culinary, medicinal, dyeing and other properties across the world ([Chaveerach et al., 2008](#page-6-0)). Curcuma mangga is a species with lateral flowering habit and bold aromatic rhizomes [\(Ravindran et al., 2007](#page-7-0)). The cut rhizome emits raw mango odour and hence, the species is known as mango ginger. It was originally described from Java ([Leong-](#page-6-1)Škornič[ková et al., 2010\)](#page-6-1) and is distributed in Andaman & Nicobar Islands of India, Thailand, Indonesia and Malaysia ([Pandey and Diwakar,](#page-7-1) [2008;](#page-7-1) [Singh et al., 2016;](#page-7-2) [Singh, 2017;](#page-7-3) [Sirirugsa et al., 2007](#page-7-4)). C. amada (a centrally flowering species native to Eastern India) is also known as mango ginger due to similar aroma and is grown in different parts of India [\(Ravindran et al., 2007](#page-7-0); [Singh, 2017\)](#page-7-3).

Traditionally, rhizomes of C. mangga are used in the preparation of pickles, sauce, candy etc. apart from their use as a spice, vegetable and salad [\(Sirirugsa et al., 2007](#page-7-4); [Singh, 2017\)](#page-7-3). Curcumin obtained from Curcuma species, commonly known as Indian Solid Gold, has multifaceted applications in drug industry as anticancer, cardio-protectant, antiviral, anti-fungal, anti-allergic, antioxidant and wound healing agent besides its use as a natural colourant and preservative in food

industry [\(Aggarwal et al., 2007\)](#page-6-2). Mango ginger rhizomes with 0.18–0.47% curcumin are also an alternative source of this industrially important compound [\(Bos et al., 2007\)](#page-6-3). Crude and fractionated extracts of rhizomes have been reported to possess anti-cancer activity against six human cancer cell lines ([Malek et al., 2011](#page-6-4)). In vivo toxicity studies suggested safety of ethanolic extracts of rhizomes in tested animals and hence the species could be used as an alternative to modern medicines ([Yuandani and Suwarso, 2017\)](#page-7-5). Leaf extracts also possessed functional food properties such as antioxidant, anti-inflammatory and anti-cancer activities [\(Liu and Nair, 2012](#page-6-5)). A patent (WO2015063751 A1) has recently been granted for use of its extract in the treatment of prostate cancer. Essential oil from rhizomes contains industrially valuable β-Myrcene as major component, which has been regarded as infrageneric chemotaxonomical marker for the species ([Wahab et al., 2011\)](#page-7-6).

Despite multifaceted applications, no systematic efforts have been made to cultivate the species on large scale. Most of the raw material is contributed by the non-discrete supplies from wild, which reduces uniformity of the final product. Cultivation could provide homogeneous quality raw material and hence, standardization of suitable agro-techniques is a pre-requisite (Waman [and Bohra, 2016](#page-7-7)). Optimization of propagule size could help in saving the seed rhizome requirement

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without compromising the economic yield ([Hailemichael and Tesfaye,](#page-6-6) [2008;](#page-6-6) [Padmadevi et al., 2012](#page-7-8)). Though rhizomatous species are well studied in this regard, no attention has been paid so far in this species. Hence, the present study is the first report concerning standardization of size of seed rhizome for commercial cultivation of C. mangga. Further, profiling of essential oil from different rhizome tissues was attempted for the first time.

2. Material and methods

2.1. Experimental site

Present investigation was conducted for two years (2015–16 and 2016–17) in the Division of Horticulture and Forestry, ICAR- Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India (11°36′42.7″ N, 92°43′3.9″E). Mean minimum and maximum temperature and rainfall of the experimental site during cropping period is presented in [Table 1](#page-1-0).

2.2. Treatment details

Local collection of Curcuma mangga Val. et Zijp., maintained in the germplasm block of the institute, was used for the study. Earlier report on C. longa suggested that planting of extra-large rhizomes resulted in poor yields due to presence of secondary and tertiary rhizomes in the propagules [\(Hossain et al., 2005](#page-6-7)). Further, extra-large rhizomes are easily broken during planting which also renders them unsuitable for mechanical planting. Considering this, rhizome size was limited to 25 g in the present study. Healthy rhizomes were allowed to cure in the field and then graded into different size groups viz. 5–10 g, 10–15 g, 15–20 g and 20–25 g before planting on raised beds.

2.3. Crop management

Fields were ploughed using a tractor mounted plough to bring the soil to fine tilth followed by leveling. Raised beds of $2 \text{ m} \times 1 \text{ m} \times$ 0.15 m were prepared and well decomposed farmyard manure was applied at the rate of 15 kg/m^2 . Graded rhizomes were planted in these beds at $0.3 \text{ m} \times 0.3 \text{ m}$ spacing. Crop was grown under rainfed conditions. During each cropping cycle, two hand weedings were carried out followed by earthing up. After first hand weeding, second dose of farmyard manure was applied at the rate of 15 kg/m^2 to all the treatments. No pests or diseases were noticed in both the cropping seasons.

2.4. Record of morphometric observations

Crop was harvested when the leaves showed drying symptoms. Rhizomes were carefully uprooted from soil without damaging the clump. From each treatment, ten representative clumps were selected for recording clump weight (g), number of primary rhizomes and

number of secondary rhizomes per clump. Fifteen rhizomes from each treatment were used for recording weight (g) and length (cm) of primary and secondary rhizomes. Known amount of rhizome pieces were sliced (five replications), oven dried at 60 °C and dry recovery percentage was calculated.

2.5. Curcumin and total phenol content

Curcumin content and total phenols were determined in primary, secondary and mother rhizomes obtained from each treatment. Cured rhizomes were sliced, oven dried at 45 °C for 72 h, grinded in electric grinder and used for determination of curcumin content (%) following method described elsewhere ([Shamina et al., 2012\)](#page-7-9). Six gram dried powder was cold percolated with 30 ml methanol followed by vacuum filtration and evaporation to obtain the crude extracts, which were used for determining total phenol content. Both the analyses were performed with four replications.

2.6. Essential oil determination and GCMS analysis

For determining essential oil content, 200 g fresh rhizomes were sliced into small pieces and hydro-distillation was carried out using Clevenger apparatus for oils lighter than water. Essential oil yield (ml) was pooled from mother rhizomes, primary rhizomes and secondary rhizomes to represent the mean oil yield per plot in each treatment. Essential oils of primary, secondary and mother rhizomes were subjected for gas chromatography–mass spectroscopy (GC-MS) analysis.

The GC-MS analysis was carried out in Varian-3800 Gas Chromatograph coupled with Varian-4000 Ion-Trap Mass Spectrometer. For analysis, extracted samples of mother rhizome, primary rhizome and secondary rhizome were injected into the injector port. MS column viz. VF-5MS (Factor four) (Varian, USA) fused-silica capillary column of $30 \text{ m} \times 0.25 \text{ mm}$ id, 0.25 mm film thickness was used for the analysis. Temperature of the injector was set at 250 °C and all injections were made initially in split (1:20) mode for 0.5 min followed by split-less. Temperature of the detector was 270 °C and temperature programme for column was as followed: 40 $^{\circ} \mathrm{C}$ for 3 min at an increment of 3 $^{\circ} \mathrm{C/min}$ to 190 °C, hold for 1 min, then 5 °C/min to 220 °C and maintaining the constant temperature for 5 min.

Mass spectrometer was operated in external electron ionization mode with Helium as carrier gas (1 ml/min), 250 °C as injector temperature, 180 °C as trap temperature, 190 °C for ion source-heating, 260 °C as transfer line temperature, 70 eV as EI-mode and full scanrange (50–350 amu) was used. Total volatile production was estimated by summing all the GC peak areas in the chromatogram and individual compounds were quantified as relative percent area. The compounds were identified by comparing the retention index which was determined by using homologous series of n-alkanes (C5–C32) as standard ([Kovatz, 1965\)](#page-6-8) and comparing the spectra using two spectral libraries available as Wiley and NIST-2007.

Yield parameters in Curcuma mangga as influenced by size of seed rhizome (pooled data for 2015–16 and 2016–17).

Values followed by same alphabet in a column represent non-significant differences at 5% level of significance using least significant differences.

Fig. 1. Dry recovery (%) in different types of rhizomes (MR: mother rhizome; PR: primary rhizome; SR: secondary rhizome) as influenced by size of seed rhizome (T₁: 5-10 g; T₂: 10-15 g; T₃: 15-20 g; T₄: 20-25 g).

2.7. Statistical analysis

Data collected from field and laboratory experiments were subjected for analysis of variance using Web Agri Statistical Package Ver. 2.0 (ICAR-CCARI, Ela, India) and mean separation was performed using least significant difference. For determination of curcumin, factorial completely randomized design was adopted with type of rhizome and rhizome size as two factors, whereas completely randomized design was adopted for other quality parameters.

3. Results and discussion

3.1. Yield attributes

An effort was made to optimize the size of propagule for mango ginger cultivation that could support higher yields with better quality parameters suitable for processing and pharmaceutical industries. Results ([Table 2](#page-2-0)) suggested that larger seed rhizomes (T_3 and T_4) significantly improved yield and quality parameters when compared to other size groups studied. Yield of fresh rhizome per plant varied between 398.53 g and 695.03 g ([Table 2](#page-2-0)). Heavier propagules are known to store large quantities of food reserves that could provide necessary energy for better establishment and growth [\(Blay et al., 1998](#page-6-9); [Nybe and](#page-6-10) [Raj, 2004;](#page-6-10) [Hossain et al., 2005\)](#page-6-7) as seen in present study. Quicker sprouting and vigorous growth due to increased availability of stored reserves in the larger sized seed rhizomes has been reported in ginger ([Hailemichael and Tesfaye, 2008;](#page-6-6) [Mahender et al., 2015\)](#page-6-11). In turmeric, larger seed rhizomes were reported to have larger buds and diameter, thus producing vigorous seedlings [\(Padmadevi et al., 2012\)](#page-7-8). As C. mangga is grown as a rainfed crop, the larger seed rhizomes might have played a vital role in harnessing soil moisture and served as a storage material to support the plants under unfavourable conditions. Similar phenomenon was observed by [Hailemichael and Tesfaye \(2008\)](#page-6-6) in ginger. Hence, improved yield attributing parameters in larger seed rhizomes of C. mangga observed during the present study could be justified.

Highest yield of fresh rhizomes was recorded in T_3 (15–20 g) and further increase in seed rhizome size did not improve the rhizome yield per plant. Use of both 15–20 g and 20–25 g sized seed rhizomes was found to be equally good for improving weight of primary (39.22–39.30 g) and secondary rhizomes (19.05–22.58 g). Non-significant yield variations amongst T_3 and T_4 are in accordance with a report on turmeric, which suggested increase in yield components with increase in size of seed rhizomes only upto a certain level after which the yields stagnated [\(Hossain et al., 2005\)](#page-6-7). In case of production of primary and secondary rhizomes in each clump, all treatments except $T₂$ remained statistically on par with each other. Number of primary rhizomes per plant varied between 2.72 and 4.33, while number of secondary rhizomes varied between 13.06 and 18.28. Significantly longest primary rhizomes (12.52 cm) were obtained in plants raised using largest sized seed rhizomes, whereas the length of secondary rhizome did not vary with the treatments. In general, primary rhizomes were longer, broader and heavier than the secondary rhizomes, irrespective of the treatments imparted. This could be attributed to the difference in physiological age of tissues as primary rhizomes develop earlier than the secondary rhizomes and hence, get more time for development [\(Hossain et al., 2005](#page-6-7)).

3.2. Dry recovery (%)

Dry recovery is an important parameter in medicinally important species as most of them are traded in dried form. In general, dry recovery trend was found to be in the order of mother rhizome $(30.33%)$ > primary rhizome $(19.80%)$ > secondary rhizome $(17.44%)$ ([Fig. 1](#page-2-1)). The recovery was significantly influenced by the size of seed rhizome as smaller sized rhizomes were found to have lower dry recovery. In mother rhizome, highest recovery of dry biomass (34.64%) was recorded from largest sized propagules studied (T_4) . This remained

CD: critical difference.

** Significant at 1% level of significance.

Fig. 2. Total phenolic content (mg/g GAE) in different types of rhizomes (M: mother rhizome; P: primary rhizome; S: secondary rhizome) as influenced by size of seed rhizome (T₁: 5-10 g; T₂: 10-15 g; T₃: 15-20 g; T₄: 20-25 g).

Fig. 3. Mean essential oil yield (ml) per plot (2 m^2) as influenced by size of seed rhizome.

statistically similar with rhizomes obtained from propagules of 10–15 g size (T_2) . In primary rhizome, maximum recovery (21.65%) was recorded in T_2 , but increase in size of seed rhizome could not influence the recovery and the value remained statistically similar between T_3 and T4. Treatments had no influence on the dry recovery percentage in case of secondary rhizomes. Dry matter recovery in C. mangga in the present study was higher than that in earlier reports on another related

species viz. C. amada i.e. 14.93% ([Sajitha et al., 2014](#page-7-10)) and 18.34% ([Kharade et al., 2017](#page-6-12)). However, in these studies, type of rhizome used for estimating dry recovery percentage was not mentioned.

3.3. Curcumin content (%)

Curcumin is one of the most valued components of medicinal importance that has been reported to occur in a number of Curcuma species. Size of seed rhizome, type of harvested rhizome and their interaction revealed highly significant differences for curcumin content ([Table 3\)](#page-3-0). Except for T_2 (10–15 g), concentration of curcumin increased with increase in size of seed rhizome. As high as 0.46% curcumin was reported in rhizomes obtained from largest sized propagule. In case of rhizome type, secondary rhizomes had highest content of curcumin (0.45%), followed by mother rhizomes (0.35%) and primary rhizomes (0.34%). The interaction effect exhibited significant differences amongst various treatments studied and curcumin content varied between 0.18% (10–15 g + mother rhizome) and 0.50% (20–25 g + mother rhizome). A number of treatment combinations exhibited curcumin content that was on par with the best treatment $(T_4 + \text{mother})$ rhizome).

In general, mother rhizome, primary rhizome and secondary rhizome are known to differ morphologically, biochemically and physiologically, which might have caused variations in curcumin content in

Volatile composition of mother rhizome (MR), primary rhizome (PR) and secondary rhizome (SR) of Curcuma mangga as revealed by GC–MS analysis.

present study. However, an earlier report on turmeric suggested that use of different types of propagules had no influence on curcumin content ([Kumar and Gill, 2010](#page-6-13)). Each species might have a specific pattern of curcumin accumulation, which probably contributed to contrasting results observed in present study. Such species specific variations for curcumin accumulation at different phenological stages have been reported in C. amada and C. aromatica ([Sajitha et al., 2014](#page-7-10)). Genetic differences are also known to influence physiological and biochemical processes and hence curcumin content varies greatly among the Curcuma species. Some of them contain relatively higher levels of curcumin e.g. C. longa (5.83%), C. aromatica (1.25%) etc. whereas other may contain moderate (C. caesia: 0.14%, C. amada: 0.21% etc.) or low (C. angustifolia: 0.03%) levels of this compound [\(Shamina et al., 2012](#page-7-9); [Kharade et al., 2017](#page-6-12)).

3.4. Total phenolic content (mg/g gallic acid equivalent (GAE))

Total phenolic content was determined in mother rhizomes, primary rhizomes and secondary rhizomes obtained from each treatment, which revealed significant differences. In general, primary rhizome had higher content of total phenols than other tissues, whereas, lowest values were noticed in the secondary rhizomes irrespective of the treatment employed. Highest total phenolic content (41.95 mg/g gallic acid equivalent) was recorded in mother rhizomes of 15–20 g sized propagules ([Fig. 2](#page-3-2)), which was followed by primary rhizomes of T_3 (37.16 mg/g GAE) and T_4 (35.84 mg/g GAE). Interestingly, lowest content (6.17 mg/ g GAE) of total phenols was obtained in secondary rhizomes of T_3 in which highest values for total phenolic content were noticed for mother rhizomes and primary rhizomes. Six phenolic compounds viz. Gallic acid, Catechin, Epicatechin, Epigallatocatechin, Epigallatocatechingallate and Gallocatechingallate have been identified in the fresh rhizomes of C. mangga [\(Pujimulyani et al., 2013](#page-7-11)), which are known to contribute to the antioxidant activity of the species. They have reported total phenolic content of about 40 mg GAE per 100 g in samples collected from Indonesia, which is lower than present findings mainly due to the fact that total phenolic content determination was done in dried tissues in present study as against fresh tissues in Indonesian study.

3.5. Essential oil yield (ml/ plot)

A large number of species including rhizomatous species are known to contain essential oils, which are generally extracted by distillation process. Such oils are valued for various flavor, fragrance and drug industries. Oil yield from C. mangga varied with the size of seed rhizome and use of larger size rhizome for planting yielded highest quantity of oil per unit area in the present study. Essential oil yield is the product of oil content and rhizome yield obtained in a particular treatment. Progressive increase in the essential oil quantity was evidenced with increase in size of seed rhizome used for planting ([Fig. 3](#page-3-3)). As high as 45.05 ml of oil/ plot was obtained from the treatment involving use of 20–25 g seed rhizome, whereas significantly lower yield of 27.32 ml was obtained with use of lightest propagules tested. In ginger, increase in size of propagule resulted in corresponding increase in oil recovery ([Mahender et al., 2015](#page-6-11)). Oil content is reported to vary greatly among the species e.g. 1.83% in ginger [\(Mahender et al., 2015](#page-6-11)), 2.10% in C. amada and 5.20% in C. aromatica ([Sajitha et al., 2014\)](#page-7-10) as against 0.37% in C. mangga in the present investigation. C. mangga grown in Malaysia was found to have only 0.12% essential oil content, which could possibly be attributed to differences in the genotypes, growing environment and extraction conditions [\(Kamazeri et al., 2012\)](#page-6-14).

3.6. GC–MS analysis of essential oil

Analysis of essential oils using GC–MS resulted in identification of 59 (mother rhizome), 60 (primary rhizome) and 58 (secondary rhizome) major constituents which contributed to 98.01%, 97.64% and

Fig. 4. GC–MS chromatogram of essential oil from C. mangga mother rhizome.

Fig. 5. GC–MS chromatogram of essential oil from C. mangga primary rhizome.

97.39% of total components, respectively. These compounds were present in varied concentrations in the tissues studied [\(Table 4](#page-4-0); [Figs. 4](#page-5-0)–6). Irrespective of the kind of tissue used for analysis, β- Myrcene was the most dominant constituent contributing to 52.357% (mother rhizome), 58.533% (primary rhizome) and 65.331% (secondary rhizome) of essential oil composition of mango ginger in present investigation. These findings are in conformity with previous report from Malaysia by [Wahab et al. \(2011\)](#page-7-6) who reported Myrcene (46.5%) as major constituent. Another report by [Wong et al. \(1999\)](#page-7-12) from Malaysia also suggested dominance of β- Myrcene in essential oil obtained from C. mangga with percentage area of 79%. Variations in % area of these compounds in different reports could be attributed to differences in agro-climatic conditions, genotype, harvesting stage, tissue and method used for oil extraction etc. [\(Kamazeri et al., 2012](#page-6-14); [Wahab et al.,](#page-7-6) [2011\)](#page-7-6).

β-Myrcene is commonly used in the commercial production of flavor products, cosmetics, soaps, air fresheners and as raw material for finer grade perfumery. Studies have been made to ascertain the toxicity reaction of β-Myrcene using animal models. The experiments found no maternal toxicity or visible malformations even at the highest dose tested (500 mg/kg) in Wistar rats. No-observed-adverse-effect level (NOAEL) for toxicity on fertility and reproduction was found to be 300 mg of β-Myrcene/kg body weight, when administered orally ([Paumgartten et al., 1998](#page-7-13)). However, the material data safety sheet of this compound indicated possibility of skin, eye and respiratory irritation, if the body parts are exposed (Sigma-Aldrich, MSDS, Ver. 5.0). Acute toxicity of Myrcene was found to be low in rodents on oral administration with approximate lethal doses of 5.06 g/kg body weight for mice and 11.39 g/kg body weight in case of rats ([Paumgartten et al.,](#page-7-14) [1990\)](#page-7-14).

Fig. 6. GC–MS chromatogram of essential oil from C. mangga secondary rhizome.

Thirteen, ten and nine compounds were in the concentration above 1% in essential oils of mother, primary and secondary rhizomes, respectively. Cyclofenchene was second most abundant compound and the content ranged between 6.444% and 10.662% amongst the tissues tested. The common dominant compounds in all three types of rhizomes were 3-Bornanol; trans-Ocimene; α-Pinene; α-Himachalene, Androstane-3,16-diol; (5α,9α,10β)-Kaur-15-ene; β-Pinene and 12-hydroxy- (12β)-Androst-4-ene-3,17-dione. Apart from these, Germacrone; 3-Terpinolenone; trans-6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5 isopropenyl-Benzofuran; Androsterone were also dominant compounds found only in oil of mother rhizomes. (+)-Epi-bicyclosesquiphellandrene and (5α)-Androst-1-en-3-one were typically present in primary rhizomes, whereas Camphene was exclusive dominant component in secondary rhizomes. Minor constituents of essential oil from C. mangga have been reported to have synergistic effects on their antimicrobial properties [\(Kamazeri et al., 2012](#page-6-14)). The compound Elixene was found only in primary rhizome whereas α-Cadinene was absent in secondary rhizome. Considering the variations in essential oil composition in three tissue types studied, the suitable tissue type could be selected for industrial use as per the requirement of specific compound.

4. Conclusion

Curcuma mangga is an underutilized yet potential species of economic importance and hence systematic cultivation is required. Present was the pioneering attempt in this regard, which revealed significant role of selection of seed rhizome size to obtain optimum yield with desired quality. Seed rhizomes of 20–25 g size were found to be optimum for producing raw material for fragrance and pharmaceutical industries, whereas smaller seed rhizomes (15–20 g) were suitable for produce meant for processing and value addition. β-Myrcene was reported to be major compound in essential oil from all three types of tissues studied, with varied proportion. Considering distinct differences in the essential oil composition from mother rhizomes, primary rhizomes and secondary rhizomes, grading of the produce could be done in order to obtain higher recoveries of intended compounds from the oils.

Conflict of interest

The authors declare that they have no conflict of interest.

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