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Identification of suitable *Myristica* species/related taxa as rootstock to combat drought in nutmeg

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ABSTRACT

A study was undertaken with the objective of identifying *Myristica* species which can withstand water scarce condition, and can be used as rootstock for grafting. Water stress was imposed on one-year-old potted plants by withholding irrigation till the plants wilted. Relative water content, chlorophyll content and protein content had significant positive correlation while membrane leakage had significant negative correlation with days taken for wilting. Ranking of species/genus *viz., Myristica fragrans, M. beddomeii, M. malabarica, Knema andamanica* and *Gymnocranthera canerica* was done based on these correlated parameters and also days taken for wilting. *M. malabarica* ranked first in terms of water stress tolerance, while *M. fragrans* and *G. canerica* ranked last and were termed as susceptible. Results of the study suggest that *M. malabarica* can be utilized as rootstock for nutmeg to combat drought.

Key words: *Myristica*, relative water content, chlorophyll, protein, catalase, peroxidase.

INTRODUCTION

Myristica fragrans Houtt. (*Myristica*ceae), commonly known as nutmeg, is an important tree spice which produces two separate spices namely, nutmeg and mace which has enormous use in the food industry. Both nutmeg and mace are also valued equally as a medicinal plant as it has immense potential in the pharmaceutical industry. Nutmeg and mace is a stimulant, aphrodisiac and is used commonly in oriental medicine for treatment of rheumatism, nausea, diarrhea, flatulent dyspepsia and dysentery. The seed contains anti-fungal, anti-infectious and anti-bacterial agents as well as volatile and non-volatile oil. The non-volatile oil is used in pharmaceuticals, cosmetics, skin care products, insect repellants, aromatic candles and soap, while the volatile oil is used in many sedative and antiseptic preparations, inhalants and chest rubs. Nutmeg thrives well in warm humid conditions in locations with an annual rainfall of 150 cm and dry conditions are not good for its growth. It requires irrigation and shade during summer months and is unable to withstand drought. Being a crop, which requires large quantity of water, rootstock which can withstand drought or require less water would be ideal to solve the problem to a great extent in nutmeg, which is propagated vegetatively through grafting. In this regard, it is better to have rootstock tolerant to drought on which high yielding nutmeg can be grafted to obtain higher productivity under water limited environment. No reports are available on drought tolerant rootstocks in nutmeg. Hence, in this study, attempts were made to identify drought tolerant rootstocks for grafting nutmeg. Drought tolerant rootstocks are utilized for crop improvement in other horticultural crops (Fernandez *et al.*, 5; McCarthy *et al*., 11; Mendes da Gloria *et al*., 12).

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MATERIALS AND METHODS

Five rootstocks belonging to related species/ genera namely, *Myristica fragrans, M. beddomeii, M. malabarica, Knema andamanica* and *Gymnocranthera canerica* conserved at Indian Institute of Spices Research, Calicut were selected for the study. The seedlings of selected *Myristica* species/genus were planted in pots and allowed to establish for one year. The potting mixture contained forest soil, sand and farm yard manure in 3:1:1 proportion. Moisture stress was imposed by withholding irrigation. One set of irrigated plants were also maintained which served as control. All plants were of uniform age at the time of stress induction. The stress treatment continued till the plants started showing wilting symptoms. Before induction of stress, plants were irrigated to field capacity daily. Observations on soil moisture content, days taken for wilting, relative water content (RWC), cell membrane leakage, total soluble proteins, total chlorophyll, proline, and peroxidase and catalase activities were recorded under control condition (irrigated), and 5 and 10 days after stress induction. RWC and cell membrane leakage were calculated as per the standard procedures. All extractions were done under ice-cold conditions using a pre chilled pestle and mortar. The procedure used by Dhindsa *et al.* (3) was followed with minor modifications for enzyme extraction. Matured leaves ($3^{rd}/4^{th}$ leaf from the tip) were collected from at least four plants from each species/genus on all observation

days and both the surfaces were cleaned thoroughly by rubbing with cotton to remove the particles adhering to the leaf surface. The leaf material was finely chopped and 0.5 g of the leaf material was ground in 5 ml of 0.1 M phosphate buffer (*p*H 7.2) containing 5 % PVP, 0.2 M ascorbic acid and 0.1 % sodium metabisulphite. The homogenate was filtered through four layers of cheese cloth and centrifuged at 10,000 rpm for 20 minutes. The supernatant was used to assay catalase and peroxidase activities.

Catalase was assayed by measuring the initial rate of disappearance of hydrogen peroxide according to Dhindsa *et al.* (3) with little modifications. The assay buffer contained 0.1M phosphate buffer (*p*H 6.8). Hydrogen peroxide (1%) was used to initiate the reaction. The enzyme activity unit was calculated based on the decrease in absorbance at 230 nm, which was measured for 90 seconds. Peroxidase activity was assayed by measuring the oxidation product of pyrogallol as per Putter (13) with suitable modifications. The assay buffer contained 0.1M phosphate buffer (*p*H 6.8) with 0.1M pyrogallol dissolved in it and was stored in amber coloured bottle to protect it from the light. Hydrogen peroxide (1%) was used to initiate the reaction. The enzyme activity was calculated based on the increase in absorbance at 420 nm, which was measured for 180 seconds. Statistical analysis was performed using MSTATC package. Cluster analysis was done using SPSS package.

RESULTS AND DISCUSSION

Soil moisture varied from 18 to 20 % during control, 14 to 16 % during 5 days and 11.5 to 13 % during 10 days after stress. The moisture content after 15 days of stress varied from 9 to 10.5 % (data not shown). Among different *Myristica* species/genus, *G. canerica* wilted very fast (11 days) followed by *M. fragrans* (12 days) while *M. malabarica* took 17 days for wilting. All species/genus had similar membrane leakage and relative water content values under well watered condition but after 5 and 10 days of stress, the values varied significantly among the *Myristica* species/genus. Relative water content decreased while membrane damage increased with stress intensity. Membrane leakage values varied from 5.6 to 6.7 % in control, 8.1 to 16.9 % after 5 days and from 10.4 to 25.6 % after 10 days of stress while relative water content ranged from 89.2 to 94.7 % during control, 55.1 to 81.4 % during 5 days and from 21.2 to 63.5 % during 10 days after stress (Table 1). *M. malabarica* had the highest relative water content and least leakage followed by *M. beddomeii* while *G. canerica* had the lowest relative water content and highest leakage values. Reduction in relative water content and increase in leakage over control (values in parentheses) was also less in *M. malabarica*. Control value represents mean of irrigated values at 0, 5 and 10 days in all the tables.

In terms of days to wilting also, *M. malabarica* took more days for wilting followed by *M. beddomeii* while *G. canerica* and *Myristica fragrans* wilted very fast. Interestingly, these two species maintained very high membrane leakage and very low relative water content values. Pigeonpea roots also showed reduced membrane integrity under water stress (Jain *et al*., 8). Utility of membrane stability as a measure of heat and drought tolerance has been demonstrated by Blum and

Table 1. Membrane leakage and relative water content of *Myristica* species/genus as affected by water stress.

Species/Genus	Membrane leakage (%)			Relative water content (%)		
	Control	5 days after stress induction	10 days after stress induction	Control	5 days after stress induction	10 days after stress induction
M. beddomeii	6.7	10.3b* (53.7)	13.7cd (104.4)	94.2	81.4a (13.6)	60.0a (36.3)
M. malabarica	6.5	8.4c (29.2)	10.4d (60.0)	90.2	77.2b (14.4)	63.5a (29.6)
K. andamanica	5.8	8.8bc (51.7)	17.2bc (196.5)	94.7	79.4ab (16.2)	51.4b (45.7)
M. fragrans	5.6	8.1c (44.6)	18.9b (237.5)	89.2	55.1c (38.2)	25.3c (71.6)
G. canerica	6.6	16.9a (151.5)	25.6a (287.9)	93.6	55.4c (40.8)	21.2c (77.4)

***** Values which do not share the same alphabets (lower case for species and upper case for stress levels) indicate that the values are significantly different from each other.
Values in the parenthesis indicate percent increase over control.

Ebercon (1) in wheat. Reduction in relative water content due to water stress has been reported in many species such as black pepper (Krishnamurthy *et al.*, 10) and fescue (Huang and Gao, 7).

Peroxidase activity varied significantly among *Myristica* species after 5 days of stress and among *Myristica* genus after 10 days of stress. The activity was significantly higher in control than under stressed condition but there was no significant difference among the stress levels. (Table 2). *G. canerica* showed highest activity during control as well as 10 days after stress while *M malabarica* showed highest activity after 5 days of stress. The activity was lowest in *Knema andamanica* both during control as well as during 5 and 10 days of stress. Though higher activities of detoxifying enzymes *viz.,* peroxidase and catalase are known to impart tolerance in many species (Chempakam *et al.*, 2; Krishnamurthy *et al*., 10; El Tayeb, 4), there was no correlation between peroxidase activity and days taken for wilting in *Myristica*; activity of catalase was very less and hence not reported here. Similar observations were made by Jain *et al*. (8) in pigeonpea and Pinheiro *et al*. (14) in *Coffea canephora* where the increase in peroxidase and catalase activities were not linked to tolerance.

Proline content varied significantly among *Myristica* species/genus. Proline content was significantly higher after 5 and 10 days of stress than control but there was no significant difference between 5 and 10 days stress on proline levels (Table 2). Highest proline content under control condition was noticed in *K. andamanica* while *M. malabarica* showed highest values during 5 days and 10 days after stress induction. Correlation analysis revealed non-significant relation between days to wilting and proline content. Increased proline content is reported during stress in some of the species such as grapes (Ramteke *et al.*, 15). Protein content was significantly higher in control than stress treatments and the content decreased with stress intensity. The values ranged from 2.04 to 2.40 during control, 1.76 to 2.14 during 5 days and from 1.65 to 2.04 during 10 days after stress respectively (Table 3). Both under control and stress conditions, highest protein was observed in *M. malabarica* followed by *M. beddomeii* while *G. canerica* had the lowest content. Percent reduction in protein content during stress was also less in *M. beddomeii* and *M. malabarica*. Ge *et al*. (6) reported decrease in soluble protein contents of leaves and roots with increasing drought in summer maize. There was no significant difference in chlorophyll content between control and stress treatments. Chlorophyll content remained stable during the stress period (Table 3). In general, *M. malabarica* maintained the highest chlorophyll content which was on par with *M. beddomeii* and *K. andamanica* while it was least in *M. fragrans* during control as well as 5 days of stress and *G. canerica* had the least value after 10 days of stress. Unlike in *Myristica*, reduced chlorophyll content was recorded during drought in some species (El Tayeb, 4; Ramteke *et al*., 15).

Correlation between days to wilting and different physiological parameters was worked out. Relative water content (r = 0.96), chlorophyll content (r = 0.73) and protein content (r = 0.86) had significant positive correlation while membrane leakage (r = -0.96) had significant negative correlation with days taken for

Table 2. Peroxidase activity and proline content of *Myristica* species/genus as affected by water stress.

Species/Genus	Peroxidase activity (a.u./mg protein)			Proline (n moles/g fresh weight)		
	Control	5 days after stress induction	10 days after stress induction	Control	5 days after stress induction	10 days after stress induction
M. beddomeii	6.8	13.0b (91.2)	15.5b (127.9)	100b	147.0c (47.0)	248.0b (148.0)
M. malabarica	6.5	16.8a (158.5)	15.6b (140.0)	105b	374.0a (256.2)	350.0a (233.3)
K. andamanica	5.0	8.7c (74.0)	8.6c (72.0)	138a	158.0c (14.5)	207.0d (50.0)
M. fragrans	5.6	8.9c (58.9)	14.8b (164.2)	58d	158.0c (172.4)	214.0cd (268.9)
G. canerica	7.9	12.7b (60.8)	19.8a (150.6)	90c	216.0b (140.0)	225.0c (150.0)

***** Values which do not share the same alphabets (lower case for species and upper case for stress levels) indicate that the values are significantly different from each other.
Values in the paranthesis indicate percent increase over control.

Table 3. Protein and chlorophyll contents of *Myristica* species/genus as affected by water stress.

Species/Genus	Protein content (%)			Chlorophyll content (mg/g fresh weight)		
	Control	5 days after stress induction	10 days after stress induction	Control	5 days after stress induction	10 days after stress induction
M. beddomeii	2.32	2.0a (11.6)	2.0a (13.8)	4.0a	4.1a	4.1a
M. malabarica	2.40	2.1a (10.8)	2.0a (15.0)	4.0a	4.0a	4.1a
K. andamanica	2.28	2.1a (8.8)	1.9ab (16.7)	3.9a	3.9a	3.8a
M. fragrans	2.11	1.8b (12.8)	1.7b (19.4)	2.7b	2.8b	2.9b
G. canerica	2.04	1.8b (13.7)	1.6b (19.1)	2.8b	2.8b	2.7c

***** Values which do not share the same alphabets (lower case for species and upper case for stress levels) indicate that the values are significantly different from each other. Values in the paranthesis indicate percent decrease over control.

Table 4. Ranking of *Myristica* species based on days to wilting and correlated parameters.

Species/Genus	Ranking as per					Mean ranking
	Days to wilt	Memb. leak	RWC	Protein content	Chlorophyll content	
M. beddomeii	2	2	2	3	2	2.2b
M. malabarica	1	1	1	1	1	1.0a
K. andamanica	3	3	3	2	3	2.8b
M. fragrans	4	4	4	5	4	4.2c
G. canarica	5	5	5	4	5	4.8c

(Values followed by lower case letters indicate mean separation by DMRT for species).

wilting. Peroxidase activity and proline content did not show any significant correlation. This suggests that these four parameters can be used to screen *Myristica* species/genus for drought tolerance. Based on the results obtained, one can fix the limit of each parameter for tolerance to drought in *Myristica* as RWC >60 % , membrane leakage <15 %, maintenance of 2 % protein and 4 mg/g fresh weight chlorophyll even after 10 days of stress. *Myristica malabarica* and *M. beddomeii* fulfill the above criteria and these can be considered as tolerant. Though it is said that nutmeg (*Myristica fragrans*) is susceptible, there are no evidences so far to show that it is really susceptible. This is the first report on the response of *Myristica* species/genus to water stress which provides evidence for the susceptibility of *Myristica fragrans* to water stress. In fact, selection for drought tolerance is usually done on the basis of rootstock resistance to water deficit in grapes. Ranking of species based on correlated parameters considered together (Table 4) suggests that *M. malabarica* ranks first in terms of tolerance, *M. beddomeii* ranks next which is on par with *K. andamanica* while *G. canerica* ranks last which is on par with *M. fragrans*. When the cluster analysis was carried out with only these correlated parameters (results not presented), *M. malabarica, M. beddomeii* and *K. andamanica* clustered together while *M. fragrans* and *G. canerica* formed a different cluster.

Results of the present study show that *M. malabarica* is tolerant to drought and it can be used as rootstock for grafting nutmeg. High yielding nutmeg varieties can be used as scion on *M. malabarica* rootstock for grafting to enhance yield as well as drought tolerance in nutmeg. Rootstocks have been utilized for drought tolerance in crops, such as apple, coffee, peach, grapes, chestnut etc. In apple, among rootstocks, M7 was tolerant to drought and frost (Senin and Senin, 16), which showed reduced chlorophyll and β carotene under drought (Sircelj and Batic, 17). Reduction in relative water content was also noticed in drought tolerant peach rootstocks (Kaynas *et al.*, 9). This is the first report on the drought tolerance studies

and also the possibility of utilizing rootstock for drought tolerance in nutmeg.

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