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*Original Research Article*

# Effects of season and spacing on growth pattern and seed yield of Muskdana genotypes (*Abelmoschus moschatus* L.) and radical scavenging activity of its seed oil

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**ABSTRACT**

This study aims to evaluate the effects of season and spacing on growth patterns and seed yield performance of muskdana by estimating fixable and non-fixable components of genetic variances for diverse traits of economic significance and identification of highly divergent clusters of genotypes to exploit them in hybridization program. Radical scavenging activity of oil from muskdana seeds was also checked. The field experiment was conducted in two seasons , namely January to June (season I) and July to December (season II) with five spacing levels treatment 1, 2, 3, 4, and CIMAP of *Abelmoschus moschatus*. From the obtained results, it can be concluded that genotypes CIM-AM 22, CIM-AM 40, and CIM-AM 49 of *A. moschatus* showed greater potential in terms of yield. *A. moschatus* oil has also effective and powerful reducing power and exhibits significant radical scavenging activity which is comparable with standard antioxidants such as ascorbic acid, BHA, and BHT.

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## 1. Introduction

Medicinal plants are potential sources of biologically active compounds with therapeutic properties. They are also rich sources in terms of metabolites such as essential oils, phenolic and organic acids and have been used in this regard from primitive times to the present (Usanmaz., 2018; Eskimez et al., 2019; Gündeşli et al., 2019). *Abelmoschus moschatus* L., commonly-known as muskdana or ambrette seed belongs to Malvaceae family and is native to India (Srivastava, 1995). It is valued for its scented seeds. The seeds of *Abelmoschus moschatus* L. yield an essential oil with a strong musky and brandy odor due to the presence of ambrettolide, a macro cyclic lactone in the seed coat (Dwivedi et al., 2013).The other major components are farnesol and farnesyl esters, acyclic aliphatic esters and terpenes. Ambrette seeds are exported to China, Germany, France, Netherlands, Nepal, Spain, Belgium, UAE, Switzerland, Singapore and The United Kingdom to the extent of about 116 quintals in a year because of its diversified uses.

From January 2014 to November 2016, China was the largest supplier of ambrette accounting for imports worth USD 4,531,262 followed by Germany and France which imported ambrette worth USD 186,855 and USD 128,081, respectively. From January 2014 to November 2016, India imported ambrette worth USD 4,939,342 with a total quantity of 327,443 kg.

The average price of ambrette per unit/kg is USD 15.08 and the average value per shipment is USD 26,556 (Anonymous, 2017a, https://www.zauba.com/importanalysis-ambrette-report.htmL). From January 2014 to November 2016, United Arab Emirates was the largest supplier of ambrette accounting for exports worth USD 1,034,106 followed by the United States and France which exported ambrette worth USD 414,684 and USD 70,648, respectively.

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In the same timeframe (January 2014-November 2016), India exported ambrette worth USD 1,600,182 with a total quantity of 46372 kg. The average price of ambrette per unit/kg is USD 34.51 and average value per shipment is USD 15,536 (Anonymous, 2017 b, https://www.zauba.com/export-ambrette-hs-code.htmL).

This species is extremely important from the therapeutic point of view. Because of their effectiveness against "*kapha*" and "*vata*," intestinal complaints, stomachic and heart disease, the aromatic seeds are valued medicinally. Ambrette seeds allay thirst and check vomiting and also considered as cardiotonic, digestive, constipating, carminative, diuretic and deodorant apart from possessing antiseptic, anti-spasmodic, and anti-vomiting properties (Pawar and Vyawahare, 2017). According to the Unani system of medicine, ambrette seeds cure urinary discharge, gonorrhea, leucoderma, and itching. Leaves and roots help to cure gonorrhea. The aqueous and ethanolic extracts of *A. moschatus* have a protective nature against fluoride-induced changes in the levels of neurotransmitters activity during pre- and post-natal fluoride exposure representing a critical role in growth and maturation of brain (Sudhakar and Reddy, 2018).

Presently, the cultivation area of muskdana in India is low but is increasing rapidly with seed exports to France, Germany, Singapore, Japan, and Spain for its use as an aromatic oil (Oudhia and Tripathi, 2001). Sowing date, as a critical factor affects both plant growth and yield depending on the prevailing environmental conditions especially temperature and relative humidity and low rainfall during seed maturity results in higher yields of quality seed of some plants, whereas high temperature and humidity increase seed susceptibility to fungal diseases (Delouche, 1980). Environmental factors have a dominating effect on the growth and yield of crops (Abdalla, 1969).

Growth duration and time to flowering strongly affect the climatic adaptation and yield potential of a crop. For an optimum yield, determination of days to sowing is considered as an important effort. Both quantitative and qualitative traits of crops depend on days to sowing, and growing season (Farrag, 1995). Muskdana plant sown in June as compared to August had good vegetative growth (Yadev and Dhankhar 2001). It was noticed that the plant grown in April as compared to March shows vigorous vegetative growth and high fruit setting (Incalcaterra et al., 2000). Good vegetative growth and pod yield mean high seed yield. A related study showed (Islam et al., 2000) that July sowings had high pod and seed yield as compared to the late August and October sowings. A number of muskdanagenotypes produced higher seed yield from June sowings as compared to the late July sowings.

The oil extracted from this crop has a great national and international demand. This increasing demand has motivated the farmers to cultivate this important medicinal crop in fairly large areas. The objectives of the current study are: i) to estimate the effect of environments, seasons and spacing on growth patterns and seed yield performance of muskdana for diverse traits of economic significance, ii) identification of highly divergent clusters of genotypes in order to exploit them in hybridization programme, and iii) to measure the radical scavenging activity of oil from muskdana seeds.

## 2. Experimental

### 2.1. Plant material

Fifty, wild/cultivated genotypes of muskdana were collected from various places in India and maintained at the national gene bank of CSIR-CIMAP Lucknow (Table 1).

### 2.2. Chemicals and reagents

Tris-HCl, ascorbic acid, glacial acetic acid, sodium acetate anhydrous, Folin-Ciocalteau reagent, gallic acid, SNP (sodium nitroprusside dihydrate), PBS (phosphate buffer saline) with pH 7.4, Griess reagent butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nitro blue tetrazolium (NBT), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), riboflavin were obtained from Sigma-Aldrich.

### 2.3. Isolation of essential oil from muskdana (*Abelmoschus moschatus*) seed

The freshly harvested seed of *A. moschatus* (500 g) were collected and crushed. These crushed seeds were hydrodistilled in a Clevenger apparatus for 4 hours, for extraction of the relevant essential oil. The oils were collected, measured, and dehydrated by anhydrous $Na_2SO_4$ and stored in vials at 4 °C for cooling until further analysis. The extracted yield of oil was calculated as mL per 500 g of fresh seed material.

### 2.4. Evaluation of antioxidant and free radical scavenging activity

#### 2.4.1. DPPH radical scavenging assay

The free radical scavenging activity of the muskdana (*Abelmoschus moschatus*) seed oil was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Kumarswami and Satish, 2008). The DPPH free radical reacts with hydrogen donors. Initially, it is purple and upon reaction with hydrogen donors, it becomes colorless by formation of the non-radical form of DPPH. It is a discoloration assay, which was evaluated by the addition of the antioxidant to a DPPH solution in methanol. The ability to scavenge the stable free radical of DPPH was measured by monitoring the absorbance at 517 nm. *Abelmoschus moschatus* seed oil in dimethyl sulfoxide (20-100 µg/mL) was mixed with 400 µL tris HCl buffer (100 mM, pH 7.4) and 500 µL of DPPH (10 mM) (Bondet et al., 1997). Incubation was done for 20 minutes and the final absorbance was recorded at 517 nm using a spectrophotometer (Thermo Scientific). Percentage antioxidant activity was calculated using the formula (Eqn. 1):

Antioxidant activity (%) = [Ac − As /Ac] × 100 (Eqn. 1)

by Polat et al., 2018, where, Ac = absorbance of the con-

**Table 1**
Origin/places of collection of fifty genotypes of muskdana (*A. moschatus*) maintained at CSIR-CIMAP, Lucknow.

| S.No. | Accession code | Details | Origin |
|---|---|---|---|
| 1 | CIM-AM 1 | Yellow flower | Lucknow local, Uttar Pradesh, India |
| 2 | CIM-AM 2 | Yellow flower | Bijnaur, Uttar Pradesh, India |
| 3 | CIM-AM 3 | Yellow flower | CSIR-CIMAP, Lucknow, India |
| 4 | CIM-AM 4 | Yellow flower | CSIR-CIMAP, Lucknow, India |
| 5 | CIM-AM 5 | Yellow flower | CSIR-CIMAP, Lucknow, India |
| 6 | CIM-AM 6 | Yellow flower | New Delhi, India |
| 7 | CIM-AM 7 | Yellow flower | Jorhat, Assam, India |
| 8 | CIM-AM 8 | Cream coloured flower | Mandsour, Madhya Pradesh, India |
| 9 | CIM-AM 9 | Light yellow early flower | CSIR-CIMAP, Lucknow, India |
| 10 | CIM-AM 10 | Light yellow | Lucknow,Uttar Pradesh, India |
| 11 | CIM-AM 11 | Yellow | Madhya Pradesh, India |
| 12 | CIM-AM 12 | White | CSIR-CIMAP, Lucknow, India |
| 13 | CIM-AM 13 | Yellow | Rajasthan, India |
| 14 | CIM-AM 14 | Yellow | Earnaculum, Kerala, India |
| 15 | CIM-AM 15 | Yellow | Rajasthan, India |
| 16 | CIM-AM 16 | Cream coloured flower | Chennai-Andhra Pradesh, India |
| 17 | CIM-AM 17 | Light yellow late flower | Krishnanagar, West Bengal, India |
| 18 | CIM-AM 18 | Light yellow flower | Rajasthan, India |
| 19 | CIM-AM 19 | Purple flower | New Delhi, India |
| 20 | CIM-AM 20 | Light yellow | CSIR-CIMAP, Lucknow, India |
| 21 | CIM-AM 21 | Yellow, high oil | Dwarika, Gujarat, India |
| 22 | CIM-AM 22 | Yellow, high seed yielder | CSIR-CIMAP, Lucknow, India |
| 23 | CIM-AM 23 | Yellow | CSIR-CIMAP, Lucknow, India |
| 24 | CIM-AM 24 | Yellow | CSIR-CIMAP, Lucknow, India |
| 25 | CIM-AM 25 | Yellow | CSIR-CIMAP, Lucknow, India |
| 26 | CIM-AM 26 | Cream coloured | CSIR-CIMAP, Lucknow, India |
| 27 | CIM-AM 27 | Yellow | CSIR-CIMAP, Lucknow, India |
| 28 | CIM-AM 28 | Light yellow | CSIR-CIMAP, Lucknow, India |
| 29 | CIM-AM 29 | Yellow | CSIR-CIMAP, Lucknow, India |
| 30 | CIM-AM 30 | Cream coloured | CSIR-CIMAP, Lucknow, India |
| 31 | CIM-AM 31 | Yellow | CSIR-CIMAP, Lucknow, India |
| 32 | CIM-AM 32 | Yellow | CSIR-CIMAP, Lucknow, India |
| 33 | CIM-AM 33 | Yellow | CSIR-CIMAP, Lucknow, India |
| 34 | CIM-AM 34 | Yellow | CSIR-CIMAP, Lucknow, India |
| 35 | CIM-AM 35 | Yellow | CSIR-CIMAP, Lucknow, India |
| 36 | CIM-AM 36 | Yellow | CSIR-CIMAP, Lucknow, India |
| 37 | CIM-AM 37 | Cream coloured | CSIR-CIMAP, Lucknow, India |
| 38 | CIM-AM 38 | White coloured | CSIR-CIMAP, Lucknow, India |
| 39 | CIM-AM 39 | Yellow | CSIR-CIMAP, Lucknow, India |
| 40 | CIM-AM 40 | Yellow, high seed yielder | CSIR-CIMAP, Lucknow, India |
| 41 | CIM-AM 41 | Yellow | CSIR-CIMAP, Lucknow, India |
| 42 | CIM-AM 42 | Yellow | CSIR-CIMAP, Lucknow, India |

Table 1 Continued

| S.No. | Accession code | Details | Origin |
|---|---|---|---|
| 43 | CIM-AM 43 | Yellow | CSIR-CIMAP, Lucknow, India |
| 44 | CIM-AM 44 | Yellow | CSIR-CIMAP, Lucknow, India |
| 45 | CIM-AM 45 | Yellow | CSIR-CIMAP, Lucknow, India |
| 46 | CIM-AM 46 | Yellow | CSIR-CIMAP, Lucknow, India |
| 47 | CIM-AM 47 | Yellow | CSIR-CIMAP, Lucknow, India |
| 48 | CIM-AM 48 | Yellow | CSIR-CIMAP, Lucknow, India |
| 49 | CIM-AM 49 | Yellow, high seed yielder | CSIR-CIMAP, Lucknow, India |
| 50 | CIM-AM 50 | Yellow | CSIR-CIMAP, Lucknow, India |

trol and As = absorbance of the sample.

### 2.4.2. Nitric oxide scavenging assays

The scavenging effects of extracts on NO were measured according to the method of Marcocci et al. (1994) with little modifications. Nitric oxide (NO) was generated from sodium nitroprusside (SNP) which was measured by the Griess reagent. SNP in an aqueous solution at physiological pH spontaneously generated NO by interacting with oxygen to produce nitrite ions which was estimated using Griess reagent. Scavengers of NO competed with oxygen giving rise to a small production of NO. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dichloride was read at 546 nm. *A. moschatus* seed oil in DMSO (20 to 100 µg/mL) was mixed with 200 µL of SNP (10 mM) in phosphate buffer saline (PBS) at 24 ºC for 30 min. Then, Griess reagent was mixed with 50 µL of the incubated solution. The same reaction mixture without the oil, but the equivalent amount of DMSO served as the control. Nitrite formation was inhibited by the plant extracts and the standard antioxidant ascorbic acid was calculated relative to the control (Marcocci et al., 1994). The absorbance of the resulting solution was measured at 546 nm and the corresponding percentage inhibition calculated as (Eqn. 2):

IP = [(ΔA546 of control-ΔA546 of sample)/ΔA546 of control] × 100 (Eqn. 2)

### 2.4.3. Ferric ion reducing antioxidant power (FRAP) method

The FRAP assay was done according to Benzie and Strain (1996) with little modifications. FRAP assay depends upon the reduction of ferric tripyridyl-triazine (Fe(III)-TPTZ) complex to the ferrous tripyridyl triazine (Fe(II)-TPTZ) by a reductant at low pH. (Fe(II)-TPTZ had intensive blue colour which was monitored at 593 nm (Benzie and Strain 1996). The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3.6H_2O$ solution (10:1:1). *A. moschatus* oil (50-500 µL) in DMSO was mixed with 1.5 mL of freshly prepared FRAP reagent. Incubation was done for 5 minutes at 37 ºC. Readings of the colour product (ferrous tripyridyltriazine complex) were taken at 593 nm. The results obtained were expressed as mM (Fe(II)/g) dry mass.

### 2.4.4. Total phenolic content determination

The Folin-Ciocalteau assay was used to determine phenolic content spectroscopically at 765 nm according to Mertoglu et al. (2019) with some modifications. The method is based on the principle of transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes. The *A. moschatus* oil was dissolved in DMSO. Aliquots samples (50-500 mL) were mixed with 10-fold-diluted Folin-Ciocalteu reagent (250 mL) along with 250 mL of sodium carbonate (7.5%) and incubated at 37 ºC for 90 min. The absorbance was measured at 765 nm. A mixture of DMSO and reagents were used as a blank. The final results were expressed as gallic acid equivalents using a linear equation of y=0.007x+0.268, $R^2$=0.994. Gallic acid (0-200 µg/mL) was used for the construction of the standard curve. The total phenolic content was determined as milligram gallic acid equivalent (mg GAE)/g the dry weight of the sample.

### 2.4.5. Determination of radical scavenging activity in the linoleic acid system by the ferric thiocyanate (FTC) method

The radical scavenging activity of *A. moschatus* oil on inhibition of lipid peroxidation was measured according to ferric thiocyanate assay (Takao et al., 1994), with little modifications. The reaction mixture containing 0.2 mL of 100 µg/mL of *A. moschatus* oil, 0.2 mL of linoleic acid emulsion (25 mg/mL in 99% ethanol) in 17.5 mg tween 20 as an emulsifier and 0.4 mL of 0.04 M phosphate buffer (pH 7.0), was incubated in the darkness at 40 ºC for 30 min. A 0.1 mL aliquot of the reaction mixture was then added to 3 mL of ethanol 70% (v/v), 100 µL of freshly prepared ammonium thiocyanate (30% w/v) and 100 µL of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid. The absorbance of the resulting red colour

was measured at 500 nm. Aliquots were assayed every 10 h until the day after the absorbance of the control solution (without the plant extract) reached maximum value. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were used as positive control. All determinations were performed in triplicate (n = 3).

#### 2.4.6. Experimental site and observations

The genotypes were grown at the experimental farm of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow India, during two consecutive vegetation seasons in the years 2015-2016 under normal fertility conditions. Seeds were sown at different spacing, namely Treatment 1 (CIM AM 22)-50 cm × 30 cm; Treatment 2 (CIM AM (25)-50 cm × 45 cm; Treatment 3 (CIM AM 5)-50 cm × 60 cm; Treatment 4 (CIM 40)-60 cm × 60 cm; Treatment 5 (CIM AM 49)-75 cm × 60 cm. The observations were done regarding growth characters, namely fruit length at maturity, fruit diameter at maturity, first fruit-producing node, plant height, length of immature fruit, the diameter of immature fruit, number of seed per fruit, fruit number on the main stem, fruit number on branches, total fruit number per plant, yield per plant growth characteristics.

#### 2.4.7. Statistical analysis

The split plot design in four replications as the main plot and the three sowing dates as subplot were used. The experiment was done in a randomized bock design with four replications. The data were analyzed using MSTAT-C statistical software (version 3). Cluster analysis and correlation analysis was done using R-software (version 3.2.2) and SPSS (version 23), respectively to determine percentage contribution to total diversity and correlation coefficient among the characteristics (Panse and Sukhatme, 1978).

## 3. Results and Discussion

### 3.1. Effect of season and spacing on the growth pattern of muskdana genotypes

Late sowing on 20$^{th}$ July and 10$^{th}$ August had significant negative effects on plant height of genotypes at 50% flowering in both years. The highest plants and number of leaves of genotypes were obtained at early sowing on 1$^{st}$ July. The reductions in plant height of genotypes were 14% and 32% due to the late sowing on 20$^{th}$ July and 10$^{th}$ August, respectively. It shows that degree of branching and growth habit of muskdana genotypes were highly variable. About 59% of genotypes were erect type, while 24.7% genotypes were characterized with medium growth and 16.4% of genotypes with the procumbent type of growth habits. Regarding the branching habit of genotypes, 41.1% had strong branching habits, while 25% were without branches. There were 30.1% of the genotypes with smooth glabrous stem, while 54.1% of the muskdana collections had slight pubescence and rest 15.1% were characterized with conspicuous pubescence on stems. Similarly, the extent of pubescence varies on leaves and fruits of muskdana. Thirty percent of muskdana genotypes were smooth, while 35% were rough and prick type of pubescence.

The data pertaining to the days taken for first flowering and 50% flowering were found to differ significantly among the various treatments (Table 2). With regard to pod yield per plot and pod yield per hectare, significant differences were observed among the treatments (Table 3). The results revealed that significant differences existed among the various treatments with regard to pod characters. Mature fruit length had a positive and significant correlation ($P \leq 0.05$) with fruit length of immature fruit ($r$ = 0.78) and number of seed per fruit ($r$ =0.443); but negatively correlated with the first fruit-producing node ($r$ = -0.299) and mature fruit diameter ($r$ = -0.027) (Table 4). In season I, the spacing of 60 × 60 cm took the least days (102.31 and 80.22 for 50% flowering and first flowering, respectively) followed by the spacing of 60 × 45 cm, which recorded 105.20 days for 50 percent flowering and 83.40 days for first flowering. The widest spacing (75 × 60 cm) took longer days (116.30 and 96.21 for 50 percent flowering and first flowering, respectively). In season II also, a similar trend was noticed with regard to flowering.

Among the different spacing levels, it was observed that 60 × 60 cm recorded early flowering (95.40 and 73.50 days for 50% flowering and first flowering, respectively) followed by 50 × 45 cm which took 99.21 days and 77.50 for 50 percent flowering and first flowering, respectively. The widest spacing of 75 × 60 cm recorded the maximum days (112.21days and 88.52 for 50% flowering and first flowering, respectively). Analysis of the pooled data revealed that 60 × 60 cm spacing recorded earlier flowering, whereas the wider spacing levels registered larger days for flowering. In season I, among the spacing levels, 60 × 60 cm (Treatment 3) recorded the highest number of pods per plant (25.31) and pod yield per plant (48.09 g) followed by 50 × 45 cm (Treatment 2) which registered 19.34 pods per plant and 35.01 g of pod yield per plant. The widest spacing of 75 × 60 cm (Treatment 5) recorded the least values (11.11 pods per plant and 18.00 g of pod yield per plant) which were at par with treatment T4.

In season II also, a similar trend was noticed with Treatment 3 (60 × 60 cm) registering the highest values for the number of pods per plant (28.22) and pod yield per plant (55.88 g). The next best values (22.02 pods per plant and 40.30 g of pod yield per plant, respectively) were recorded in Treatment 2 (50 × 45 cm). The least values for the number of pods per plant (13.19) and pod yield per plant (22.09 g) were recorded at the wider spacing of 75 × 60 cm (Treatment 5). Analysis of the pooled data revealed that 60 × 60 cm spacing recorded the maximum number of pods per plant and pod yield per plant, whereas wider spacing recorded lowest values for these traits. In season I, among the spacing levels, 60 × 60 cm (Treatment 3) recorded the highest pod yield per plot (1.586 kg) and pod yield per hectare (1321.14 kg) followed by 50 × 45 cm (Treatment 2) which recorded a 1.540 kg pod yield per plot and

**Table 2**
Effect of season and spacing on pod characters of *Abelmoschus moschatus*.

| Treatments | Number of pods/plant | | | Pod yield/plant (g) | | | Pod yield/plot (g) | | | Pod yield/hectare (kg) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Season I | Season II | Pooled | Season I | Season II | Pooled | Season I | Season II | Pooled | Season I | Season II | Pooled |
| Treatment 1 (CIM AM 22) | 13.22 | 15.01 | 14.12 | 22.08 | 25.52 | 23.8 | 1457 | 1684 | 1571 | 1213.68 | 1402.77 | 1308.64 |
| Treatment 2 (CIM AM 25) | 19.34 | 22.02 | 20.68 | 35.01 | 40.3 | 37.66 | 1540 | 1773 | 1657 | 1282.82 | 1476.91 | 1380.28 |
| Treatment 3 (CIM AM 5) | 25.31 | 28.22 | 26.77 | 48.09 | 55.88 | 51.99 | 1586 | 1844 | 1715 | 1321.14 | 1536.05 | 1428.6 |
| Treatment 4 CIM AM (40) | 11.44 | 14.02 | 12.73 | 18.65 | 23.13 | 20.89 | 485 | 601 | 543 | 404.01 | 500.63 | 452.32 |
| Treatment 5 (CIM AM 49) | 11.11 | 13.19 | 12.33 | 18 | 22.09 | 20.05 | 378 | 464 | 421 | 314.87 | 386.51 | 350.69 |
| S.E ± | 0.44 | 0.43 | 0.31 | 0.55 | 0.58 | 0.6 | 11.07 | 11.81 | 11.84 | 15.13 | 17.61 | 16.67 |
| C.D. (P=0.05) | 0.88 | 0.86 | 0.62 | 1.11 | 1.17 | 1.21 | 22.13 | 23.61 | 23.67 | 30.25 | 35.21 | 33.33 |

**Table 3**
Effect of season and spacing on seed characters of *Abelmoschus moschatus*.

| Treatments | Seed weight/pod (g) | | | Seedyield/plant (g) | | | Seed yield/plot (kg) | | | Seed yield/hectare (kg) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Season I | Season II | Pooled | Season I | Season II | Pooled | Season I | Season II | Pooled | Season I | Season II | Pooled |
| T1 | 0.85 | 0.88 | 0.87 | 11.24 | 13.06 | 12.15 | 0.742 | 0.86 | 0.802 | 618.09 | 718.05 | 668.07 |
| T2 | 1.02 | 1.05 | 1.04 | 19.73 | 23.12 | 21.43 | 0.868 | 1.02 | 0.943 | 723.04 | 847.16 | 785.53 |
| T3 | 1.1 | 1.21 | 1.16 | 27.84 | 34.15 | 31.17 | 0.919 | 1.13 | 1.029 | 765.53 | 938.79 | 857.16 |
| T4 | 0.83 | 0.85 | 0.84 | 9.5 | 11.92 | 10.71 | 0.247 | 0.31 | 0.279 | 205.75 | 258.23 | 232.41 |
| T5 | 0.81 | 0.83 | 0.82 | 9 | 11.25 | 10.13 | 0.189 | 0.24 | 0.213 | 157.44 | 196.59 | 177.43 |
| S.E.± | 0.01 | 0.01 | 0.02 | 0.8 | 0.9 | 1 | 0.015 | 0.02 | 0.017 | 12.16 | 14.85 | 14 |
| C.D. (5%) | 0.02 | 0.02 | 0.03 | 1.6 | 1.9 | 2 | 0.029 | 0.04 | 0.033 | 24.32 | 29.69 | 27.19 |

**Table 4**
Phenotypic and genotypic correlation among eleven quantitative traits of muskdana genotypes.

| Traits | FLM | FDM | FFPN | PH | LIF | DIF | NSPF | FNMS | FNB | TFN | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FLM | - | | | | | | | | | | |
| FDM | -0.027 | - | | | | | | | | | |
| FFPM | -0.299* | -0.028 | - | | | | | | | | |
| PH | -0.052 | 0.176 | 0.394** | - | | | | | | | |
| LIF | 0.780** | -0.092 | -0.342** | -0.115 | - | | | | | | |
| DIF | 0.1 | 0.207 | 0.079 | 0.199 | 0.121 | - | | | | | |
| NSPF | 0.443** | 0.101 | -0.217 | -0.123 | 0.440** | 0.296* | - | | | | |
| FNMS | 0.006 | -0.05 | -0.187 | -0.159 | -0.034 | -0.109 | -0.069 | - | | | |
| FNB | -0.127 | 0.283* | 0.108 | 0.182 | -0.191 | -0.12 | -0.207 | 0.504** | - | | |
| TFN | -0.076 | 0.141 | -0.087 | -0.045 | -0.133 | -0.108 | -0.188 | 0.854** | 0.863** | - | |
| YLD | 0.102 | 0.125 | -0.104 | -0.019 | 0.12 | 0.224 | -0.039 | 0.816** | 0.579** | 0.837** | - |

(*), (**) Significant at 5 and 1% levels of probability, respectively.FLM-Fruit length at maturity, FDM-fruit diameter at maturity, FFPN-first fruit producing node, PH-plant height, LIF-length of immature fruit, DIF-diameter of immature fruit, NSPF-number of seed per fruit, FNMS-fruit number on main stem, FNB-fruit number on branches, TFN-total fruit number per plant, YLD-yield per plant.

1282.82 kg pod yield per hectare. The widest spacing of 75 × 60 cm (Treatment 5) recorded the least values of 0.378 and 314.87 kg pod yield per plot and pod yield per hectare, respectively. In season II also, the same trend was noticed with the highest values for pod yield per plot (1.844 kg) and pod yield per hectare (1536.05 kg) being recorded at the spacing of 60 × 60 cm (Treatment 3). The next best values (1.773 and 1476.91 kg for pod yield per plot and per hectare, respectively) were recorded at the spacing of 50 × 45 cm (Treatment 2). The least values for pod yield per plot (0.464 kg) and pod yield per hectare (386.51 kg) were recorded in the wider spacing of 75 × 60 cm (Treatment 5).

### 3.2. Genetic correlations among different traits in muskdana

Direct selection for any given single character may increase the trait values of positively correlated characters and decline the values for negatively correlated traits as a result of correlated response to selection. The first fruit-producing node (FFPN) and maximum plant height had the strongest positive association ($r$ = 0.394). However, FFPN was highly significant and negatively correlated with immature fruit length ($r$ = -0.342). Number of seeds per fruit illustrated highly positive correlation with mature fruit length ($r$ = 0.443) and commercial fruit length ($r$ = 0.44) as well as negative association with first fruit-producing nodes ($r$ = -0.207). Fruit yield had a strong positive correlation with fruit numbers ($r$ = 0.86), but negatively correlated with other parameters. There has been reported a negative correlation among these traits. Hence, total fruit production, first fruit-producing node, and number of fruits per plant should be given more attention when being selected for higher yield and high dry matter in *A. moschatus*. Based on quantitative characters, 50 genotypes were grouped into 4 distinct clusters (Fig. 1). The first, second, third and fourth clusters respectively consist of 25 genotypes (50%), 13 genotypes (26%), 8 genotypes (16%) and only 4 genotypes (8%) out of total genotypes. The distribution pattern of genotypes into 4 clusters confirmed the existence of diversity among the genotypes (Incalcaterra et al., 2000; Islam et al., 2000; Lal, et al 2020a,b,c).

### 3.3. Radical scavenging and antioxidant property in muskdana

Numerous techniques have been used to evaluate the radical scavenging and antioxidant property of a compound. Different synthetic molecules such as DPPH, DMPD, ABTS, ferrous ion chelating activity, $H_2O_2$ scavenging activity etc. are used for the finding of natural medicinal bioactives. Flavonoids from the medicinal and aromatic plants known to be the excellent quencher of free radicals and their content varies plant to plant hence antioxidant activity varies; sometimes it is due to the synergistic property of the molecules present in the plant itself. In the antioxidant activity of *A. moschatus* oil, we evaluated the antioxidant potential of seed oil by

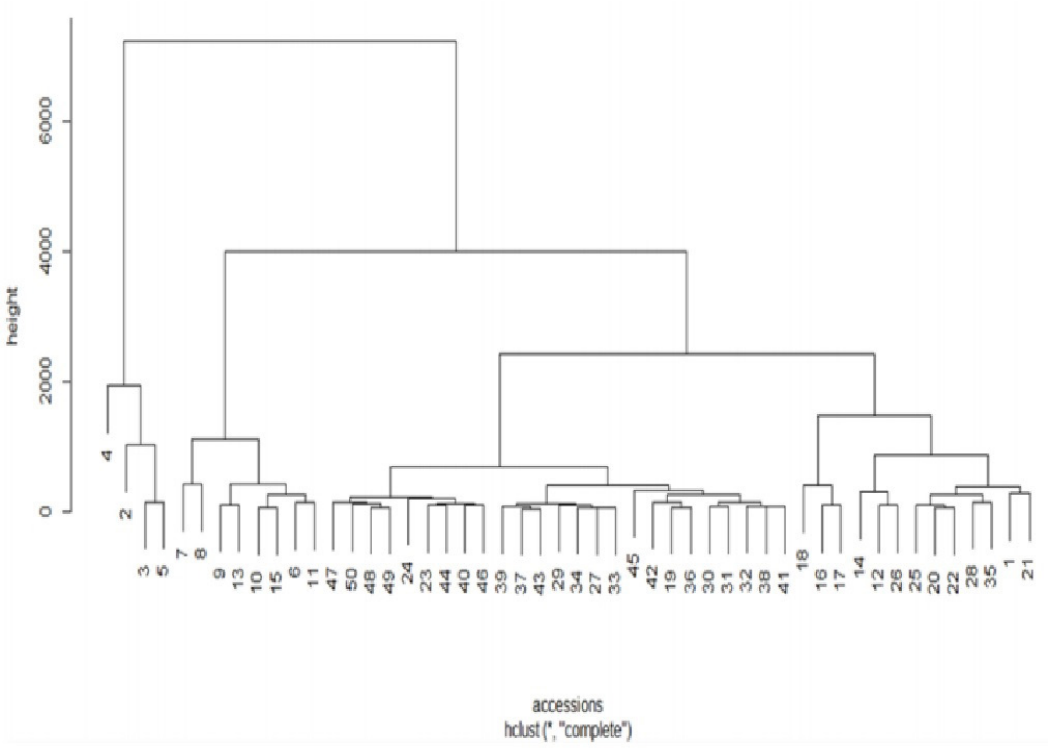


**Fig. 1.** Dendrogram of fifty genotypes of *Abelmoscus moschatus*: cluster analysis.

determination in linoleic acid emulsion system by ferric thiocyanate method were also performed. Fig. 2 depicts the scavenging activity (%) of muskdana oil along with standard antioxidants at different concentrations. The results are comparable with the standards. Nitric oxide is an important signaling molecule involved in various inflammatory processes and in cellular functions. Toxicity is mainly manifested to the conversion of superoxide radical to second reactive species peroxynitrite anion ($ONOO^-$). Furthermore, protonation leads to the formation of highly reactive product peroxynitrous acid (Balavoine and Geletti 1999). The present study assesses the role of *A. moschatus* oil at different concentrations in NO radical quenching. In this method, nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite, this ion diazotizes with sulphanilamide and naphthyl ethylene diamine forming pink colour, which was measured at 546 nm. *A. moschatus* oil restricts the nitrite formation and depicts radical scavenging property. The results are depicted in Fig. 3 and are comparable with the standards. The FRAP measures the antioxidant potential of the chemical entity in the reaction medium as reducing ability. As shown in Table 5, *A. moschatus* oil has an effective and powerful reducing power indicating remarkable antioxidant activity. $FeSO_4$ was used as a standard compound. The FRAP value calculated from the calibration curve (y = 0.012x + 0.137, $R^2$ = 0.994), was 32.54 ± 07.32 mmol Iron II per gram. Peroxidation of lipids and fatty acids leads to chains of chemical reaction which are highly damaging and are associated with biological damage.

Nevertheless, *A. moschatus* oil exhibited significant antioxidant activity comparable with the BHA and BHT and the repective results are depicted in Fig. 4. Evaluation of the antioxidant potential of *A. moschatus* seed oil was done using single electron transfer methods, viz. DPPH, and FRAP, total phenolic content, and total antioxidant capacity. During this process, determination in linoleic acid emulsion system by ferric thiocyanate method was also performed. The antioxidant activity of *A. moschatus* oil was compared to standard antioxidants such as ascorbic acid, BHA, and BHT. Radical scavenging activity is an important parameter due to the deleterious effect of radicals on the biological systems and food. DPPH radical scavenging as a chemical-based, reliable, easy to use strategy, allows a spectrophotometric-based technique for rapid analysis and determination of the oxidation endpoint. DPPH chromogen is violet in colour and attributed to single electron transfer reaction and in radical form absorbing at 517 nm (Awika et al., 2003; Yu et al., 2002). DPPH accepts hydrogen radical to become a stable diamagnetic molecule. A further outcome of this process involved the termination of the free radical chain reaction that may be otherwise very damaging (Chung et al., 2002). The role of an oxidant species is associated with the pathogenesis of aging, atherosclerosis, cancer, and neurodegenerative disorders (Perry et al., 2000).

The amount of peroxide formed during initial stage oxidation is the measure of lipid oxidation. Total antiox-

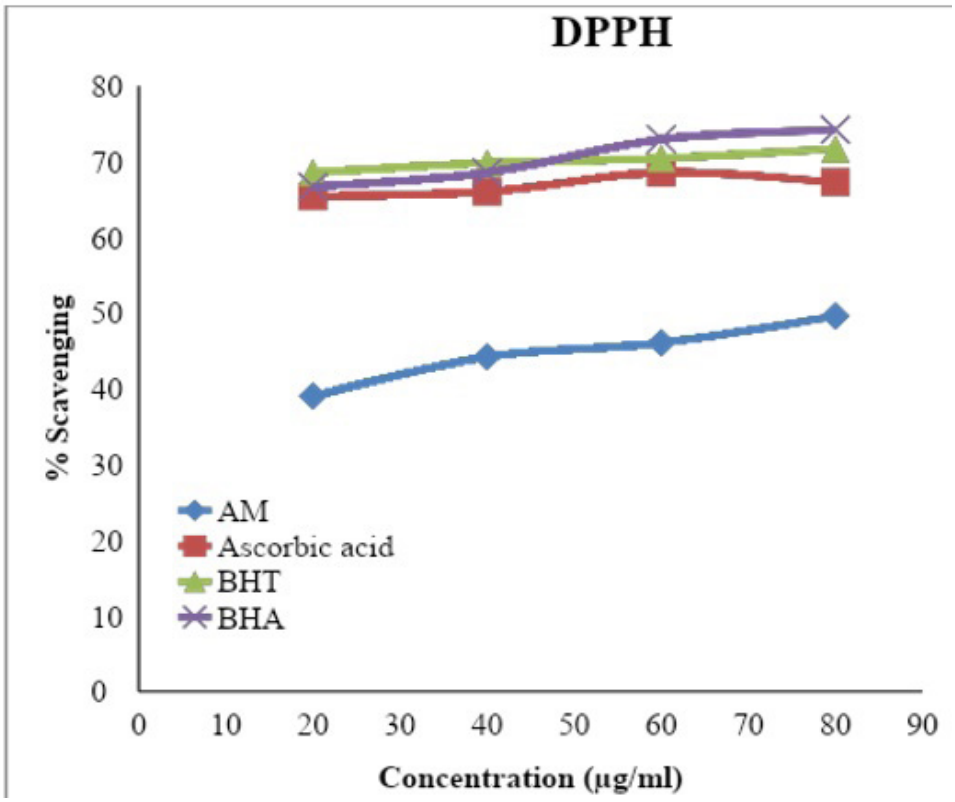


**Fig. 2.** DPPH % scavenging activity of *Abelmoschus moschatus* and standard antioxidants at different concentrations (20-100 µg/mL).

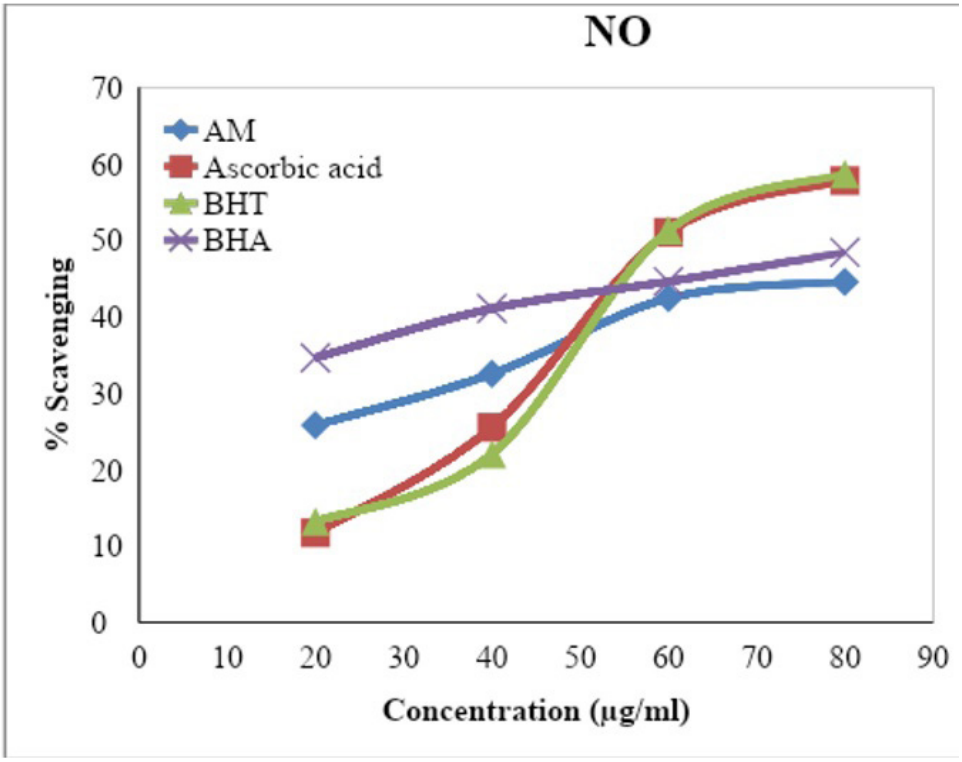


**Fig. 3.** Nitric oxide scavenging activity of *Abelmoschu smoschatus* and standard antioxidants at different concentrations (20-100 µg/mL).

**Table 5**
Total phenolic content and FRAP value of *Abelmoschus moschatus* oil.

| S.No. | Sample Name | Total Phenolic content (mg GAE/g) | FRAP value (mmol Fe II/g) |
|---|---|---|---|
| 1 | *A. moschatus* | 210.29 ± 20.41 | 32.54 ± 7.32 |

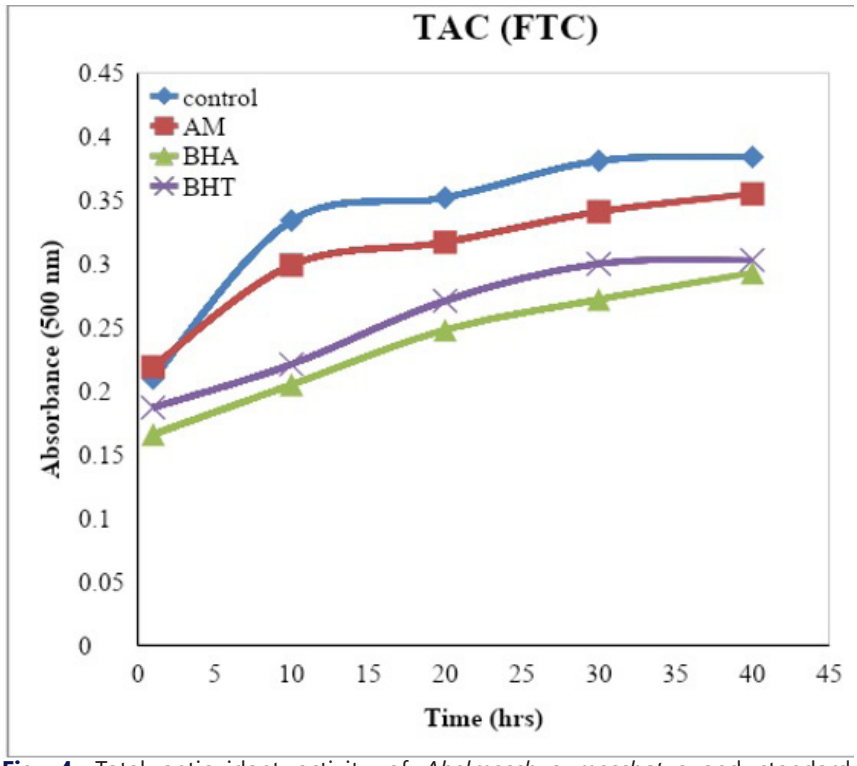


**Fig. 4.** Total antioxidant activity of *Abelmoschus moschatus* and standard antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at the same concentration 50 µg/mL assayed by ferric thiocyanate method.

idant capacity of *A. moschatus* oil in the linoleic acid emulsion and in the ferric thiocyanate system was determined using ascorbic acid, BHA, and BHT as standards. The effect of *A. moschatus* (100 µg/mL) at different time intervals (1-40 hrs) was calculated until the value of control decreased using the spectrophotometer at 500 nm. Phenolic compounds are excellent quenchers since their reduction potential (phenolic radical) is lower than that of oxygen (Soobrattee et al., 2005). Phenolic radical is less reactive capable of scavenging reactive oxygen radicals and intermediates without promoting further reactions. The total phenolic content determination was done by Folin-Ciocalteu (F-C) reagent. The total phenolic content calculated from the calibration curve ($y = 0.007x + 0.268$, $R^2 = 0.994$), was 210.29 ± 20.41 gallic acid equivalent per gram.

## 4. Concluding remarks

It was concluded in a nutshell that the genotypes CIM-AM 22, CIM-AM 40, and CIM-AM 49 showed greater potential in terms of yield attributes as they outperformed the other genotypes, indicating their usefulness as promising genotypes. The high genetic variation existed among the genotypes/germplasms in all the studied characters. *A. moschatus* oil exhibited significant antioxidant activity comparable with the BHA, and BHT. It restricts the nitrite formation and depicts radical scavenging property. The seed sowing during July month (on the first two weeks) could be recommended for Uttar Pradesh State and areas of similar conditions for the high seed yield. Early sowing (March-June) could be recommended for fresh pod production since seeds produced during July-August would be affected by rain and Yellow Mosaic Virus diseases. *A. moschatus* oil has effective and powerful reducing power which proves its potential use as a potent antioxidant activity.

## Conflict of interest

The authors declare that there is no conflict of interest.

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