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ORIGINAL ARTICLE

Effect of pre-sowing treatments with chemical mutagens on seed germination and growth performance of jamun (*Syzygium cumini* L. Skeels) under different potting substrates

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Abstract – Introduction. Jamun, propagated through seed for raising rootstock, involves a lot of cost and risk for maintenance until attainment of graftable size. Use of suitable growing substrates is essential for production of quality nursery plants. Chemical mutagenesis is a simple approach to create mutation in plants for improvement of potential agronomic traits in rootstock materials. **Materials and methods.** Seeds originating from a single 'elite' tree were soaked in 0.1 and 0.5% ethyl methane sulfonate and colchicine solutions for 24 h prior to sowing in different propagation substrates (Arka fermented coco-peat and a mixture of sand, soil and farmyard manure). Morphological, physiological and biochemical analyses were performed after 3 months. **Results and discussion.** The Arka fermented coco-peat substrate resulted in earlier and higher seed germination, higher polyembryony, greater accumulation of photosynthetic pigments and total carbohydrates, and lower synthesis of stress-induced metabolites in leaves. These performances were probably due to a better balance between the water and air capacity than in the other growth substrate. Among different doses of chemicals, colchicine at 0.1% concentration was found to stimulate early seedling emergence, improve plant morphological characters and enhance production of total chlorophyll and total carbohydrates in leaves. **Conclusion.** The graftable stage can be attained rapidly if jamun seeds are treated with 0.1% colchicine and sown in Arka fermented coco-peat substrate.

Keywords: India / Jamun / *Syzygium cumini* / rootstock nursery / seed treatment / colchicine / phenolics

Résumé – Effet des traitements pré-semis de mutagenèse chimique sur la germination des semences et la croissance des plants de jamelonier (*Syzygium cumini* L. Skeels) sur différents substrats. Introduction. Le jamelonier est traditionnellement multiplié sur porte-greffes issus de semis, ce qui engage des coûts élevés et des risques liés à l'entretien des porte-greffes jusqu'à la taille convenable au greffage. L'utilisation de milieux de culture appropriés est essentielle à la production de plants de pépinière de bonne qualité. La mutagenèse chimique est une approche simple pour créer une mutation sur les plantes porte-greffes pour en améliorer les caractéristiques agronomiques potentielles. **Matériel et méthodes.** Les graines originaires d'un seul arbre « élite » ont été trempées dans une solution de sulfonate de méthane d'éthyl ou de colchicine à 0,1 ou 0,5 % pendant 24 h avant semis sur un substrat composé d'un mélange coco-tourbe fermenté (Arka) ou d'un mélange de sable, terre et fumier de ferme (SS). Des analyses morphologiques, physiologiques et biochimiques ont été réalisées sur les plantes après 3 mois. **Résultats et discussion.** Le substrat Arka a permis une germination des graines plus précoce, une polyembryonie plus forte, davantage d'accumulation de pigments photosynthétiques et de glucides totaux que le substrat SS, et une synthèse plus faible de métabolites induits par le stress mutagène dans les feuilles des plantes. Ces performances s'expliqueraient par un meilleur équilibre entre les capacités de rétention d'air de d'eau que dans l'autre substrat. Parmi les différentes doses de produits mutagènes testées, la colchicine à 0,1 % a stimulé l'émergence précoce des semis, amélioré les critères morphologiques de croissance des plantes, et amélioré la production de chlorophylle et glucides totaux dans les feuilles. **Conclusion.** Le stade de greffage peut être atteint rapidement si les graines de jamelonier traitées à 0,1% colchicine sont semées sur substrat Arka.

Mots clés : Inde / jamelonier / *Syzygium cumini* / pépinière de greffage / semences porte-greffe / colchicine / composés phénoliques

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1 Introduction

Syzygium cumini L. Skeels (commonly named jamun, Java plum, black plum, Damson plum, Duhat plum, jambolan plum or Portuguese plum) is a large evergreen multipurpose tree of the family Myrtaceae up to 15 to 30 m in height [1]. It is native to India and thrives easily in a tropical climate. It has been used worldwide in treatment of diabetes by traditional practitioners over many centuries and has proven good anti-oxidant, anti-bacterial, antigenotoxic, anti-inflammatory and anti-HIV properties [2]. However, being an under-exploited fruit crop, it has been poorly studied in regard to genetic diversity across its distribution [3]. Though seed propagation is most common in jamun, asexual propagation such as grafting is the easiest way to preserve the particular character of the plant. However, environmental conditions such as mild temperature and humidity, a suitable grafting technique and healthy rootstock are some basic factors for development of callus tissue and forming a graft union. Most of the time, it is difficult to provide all favorable environmental conditions for grafting success in outdoor conditions in the case of jamun [4]. Thus, availability of suitable rootstock is a limiting factor for the dissemination of improved varieties of jamun.

Mutation induction technology can facilitate the development of improved varieties through improving characters of direct importance, early maturity and tolerance to biotic and abiotic stresses [5]. Thus, in the present study, an effort was made to induce variability in jamun by seed treatment with chemical mutagens. It is easy to treat the seeds with chemical mutagens which will have point mutations, rather than going for time-consuming investigation of natural diversity, as we are looking to improve specific traits such as earliness in germination, plant biomass and physiological adaptation to biotic and abiotic stress so that we can produce healthy rootstock and advance the grafting time in jamun. Thus, improvement in either a single or few economic traits and quality characters can be achieved with the help of induced mutations within the shortest possible time [6]. Chemical mutagens such as ethyl methane sulfonate (EMS), a compound of the alkaline sulfonate series, is most frequently used for chemical mutagenesis in higher plants due to its potency and the ease with which it can be used [7]. It usually causes high frequency of gene mutations and low frequency of chromosome aberrations [5]. It can proficiently induce chemical modification of nucleotides, which results in various point mutations such as nonsense, missense and silent mutations [8]. Colchicine ($C_{22}H_{25}NO_6$), originally extracted from *Colchicum autumnale*, may induce some morphological, cytological and histological changes, and even changes in the gene expression level [9]. The effect of EMS on seed germination and seedling growth has been studied in a few fruit crops such as *Carica papaya* [10] and *citrus jambhiri* [11]. Similarly, a few studies have been carried out to discover the effect of colchicine on the morphology and physiology of plants such as *Dianthus caryophyllus* [12] and *Zanthoxylum armatum* [13]. However, no research has been done so far to understand the germination, growth and physiological alterations of fruit plants such as *Syzygium cumini* upon seed treatment with chemical mutagens.

Interest in producing quality planting material by improved and modern nursery techniques has increased among nurserymen [14]. Nursery potting substrates influence the quality of seedlings produced [15]. A good growing substrate serves as a reservoir for nutrients and water, allowing oxygen diffusion to the roots and permitting gaseous exchange between the roots and atmosphere outside the root substrate [16]. Cocopeat, made from coconut husk, with a total pore space, high water retention capacity and low bulk density [20], is a good source of nitrogen, phosphorus and potassium, essential for protein synthesis and thus good seedling growth, as compared with a mixture of soil, sand and farmyard manure (FYM) [17]. Apart from improving the physical properties of the soil it also serves as a buffer against temporary drought stress and reduces the risk of plant failure during establishment [18]. Despite many advantages and availability in large quantities, it is not fully utilized for productive purposes due to its high C:N ratio (about 100:1), high content of lignin and cellulose (about 40% each), and high polyphenol content (about 100 mg 100 g^{-1} coco-peat), resulting in slow degradation under natural conditions and immobilization of plant nutrients [19]. The inhibitory effect can be eliminated by using biodegraded coco-peat [20]. Thus, fermented coco-peat can be one option for its use as a propagation substrate.

As seed germination is the first and most critical stage of plant development and the relative performance of individual plants during the early growth stage, including germination and plant establishment, can have more effects on growth and fitness [21], the present study aimed to determine the effects of pre-sowing treatment of jamun seeds with the chemical mutagens EMS and colchicine on germination, and the subsequent growth of the plant under different propagation substrates.

2 Materials and methods

2.1 Site of experimentation

The seed germination and seedling growth experiments of jamun were conducted from May to August 2013, in a shade house receiving 70% natural light at the Experimental Block of the Division of Fruit Crops, Indian Institute of Horticultural Research, Bengaluru, Karnataka, India, situated at a latitude of 13°58′ N, longitude of 78° E and an elevation of 890 m above sea level.

2.2 Seed material, chemical treatment and growth conditions

The seeds of jamun were procured from an open-pollinated seedling progeny of the Experimental Farm of the institute. The tree was 20 years old, with a medium-sized canopy with average fruit yield of 80 kg $tree^{-1}$ per annum. The average fruit weight was 9.71 g, with 3.87 cm length and 2.70 cm diameter, with total soluble solids of 13.41 °Brix. The seeds were large with an average weight of 1.39 g. The freshly extracted seeds were treated with the chemical mutagens 0.1% ethyl methane sulfonate (EMS), 0.5% EMS, or 0.1% or 0.5%

colchicine for 24 h prior to sowing. Control seeds were soaked in cold water for 24 h. Thus, there were five treatments:

T_1 = Control,
T_2 = EMS 0.1%,
T_3 = EMS 0.5%,
T_4 = Colchicine 0.1%, and
T_5 = Colchicine 0.5% .

Each treatment was replicated three times with 10 seeds per replication. The seeds were sown at 3.0 cm depth in portrays filled with two different propagation substrates; i.e., a mixture of soil, sand and farmyard manure in the proportion 1:1:1 (v/v) and Arka fermented coco-peat. The solid-state fermentation of raw coir pith was done by employing a consortium of the fungus *Aspergillus* sp. An inoculum size of 20–50% (v/v) based on the volume of the mineral medium and a substrate average particle size of 375 μm was optimum. The entire process was completed in thirty days at the nursery of the institute and labeled as Arka fermented coco-peat for its use as a propagation substrate. All portrays were covered by wire net until the end of the germination experiment so as to protect the sown seeds from rodent attack. Irrigation was done with a rose cane whenever required. The seedlings were transferred 30 days after sowing the seeds from the portrays to polybags of 10 × 8 cm (300 gauge) with two punch holes for drainage, filled with 750 g of a mix of soil:sand:FYM (1:1:1) v/v.

2.3 Measured parameters

2.3.1 Germination parameters and polyembryony

The germination percentage was calculated as percent of germinating seeds at the end of the experiment in relation to the total number of seeds sown per treatment.

The germination vigor index (GVI) was computed using the following equation, as described by Hassanein [22]:

$$GVI = \frac{x_1}{d_1} + \frac{x_2}{d_2} + \frac{x_3}{d_3} + \ldots + \frac{x_n}{d_n}$$

where $X_1, X_2, X_3, \ldots, X_n$ are the number of seeds germinated on $d_1, d_2, d_3, \ldots, d_n$ days taken for germination.

Polyembryony was calculated as the percent of seeds producing multiple seedlings in relation to the total number of seeds germinated.

2.3.2 Growth parameters

Plant height was measured from the collar region to the base of the last fully opened leaf on the main stem with the help of a measuring scale. The average number of leaves per plant was calculated by counting the number of fully opened leaves for each replication per treatment. The fresh weight of shoot and root samples was recorded separately using an electronic digital balance and then dried in an oven at a temperature of 70 °C for about four days until a constant weight was obtained. The seedling vigor index was calculated as given by Bewly and Black [23]:

$$\textit{Seedling vigor index} = \textit{Average dry weight of the seedling} \times \textit{Germination percentage}$$

2.3.3 Chlorophyll estimation

The chlorophyll contents (chlorophyll 'a', 'b' and total chlorophyll) were estimated by the method suggested by Hiscox and Israelstam [24]. Fully matured open leaves were taken as the experimental material. 100 mg of accurately weighed clean leaf sample were immersed in 10 mL dimethyl sulfoxide (DMSO), (AR grade of SRL Chem. Co., Mumbai). The samples were incubated at 70 °C for 4 h in an incubator (TH 7004, Sanco Company, New Delhi). They were then taken out, 1 mL of the solution was diluted to 5 mL with pure DMSO, and the samples were read on a UV-VIS double-beam spectrophotometer (T80+UV/VIS Spectrometer, PG Instruments Ltd.), at 470, 645 and 663 nm wavelengths using pure DMSO as a blank. Chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoids were calculated on a fresh weight (fw) basis and expressed in mg g^{-1} fw.

2.3.4 Total carbohydrate estimation

Total carbohydrates were determined using the anthrone method [25]. The weighed leaf sample (100 g) was hydrolyzed with 2.5 N hydrochloric acid in a boiling water bath for 3 h, followed by cooling and neutralizing with solid sodium carbonate, until effervescence ceased. Then the volume was made up to 100 mL and a 0.5-mL aliquot was taken and mixed with 4 mL anthrone reagent. The green to dark green-colored product was read at 630 nm on the UV-VIS spectrophotometer (T80+UV/VIS Spectrometer, PG Instruments Ltd.). The amount of total carbohydrates in the leaf sample was determined by comparing with the standard curve prepared by taking a known concentration of D-glucose in the range of 20–100 μg mL^{-1} and expressed as a percentage.

2.3.5 Total phenol estimation

Total phenols were estimated using the method of Singleton and Rossi [26]. Fresh leaf tissue (1 g) was homogenized in 5 mL of 80% methanol in a mortar and pestle three consecutive times. The extract was pooled and the volume made up to 50 mL using distilled water. Then, 0.5 mL of the sample was taken in a test tube containing 0.2 mL 1 N Folin-Ciocalteu's phenol reagent and 3.3 mL distilled water. After thorough mixing, 1 mL sodium carbonate was added and incubated at room temperature for 30 min. The blue-colored complex formed after reaction was read at 700 nm. The standard curve of phenols was prepared using gallic acid (GA) and expressed as mg GA equivalents 100 g^{-1} leaf sample.

2.3.6 Total antioxidant estimation

Total antioxidants were estimated as per the ferric reducing ability of plasma (FRAP) assay [27]. The sample was prepared by homogenizing a fresh leaf sample (1 g) in a mortar and pestle using 80% methanol 3 times. Then the extract was pooled to 50 mL volume using distilled water, and 0.2 mL of

Table I. Response of jamun to chemicals for the rate of seed germination under different propagation substrates (CP = coco-peat; SS = mixture of sand, soil and FYM in 1:1:1 ratio).

Treatment	Days for initiation of germination			Days for completion of germination			Germination index		
	CP	SS	Mean	CP	SS	Mean	CP	SS	Mean
T_1 = Control	16.00	18.67	17.34	27.67	30.67	29.17	0.393	0.342	0.368
T_2 = EMS 0.1%	15.33	20.00	17.67	25.67*	30.33	28.00	0.449	0.286	0.368
T_3 = EMS 0.5%	15.67	20.33	18.00	29.67	31.33	30.50	0.401	0.324	0.363
T_4 = Colchicine 0.1%	13.67*	18.33	16.00	27.00	28.67	27.84	0.507*	0.344	0.426
T_5 = Colchicine 0.5%	14.67	20.00	17.34	26.00	31.00	28.50	0.470	0.319	0.395
Mean	15.07	19.47		27.20	30.40		0.444	0.323	
For comparing the means of	S.E. ±	LSD at 5%		S.E. ±	LSD at 5%		S.E. ±	LSD at 5%	
Propagation Substrates (P)	2.20	0.64		1.60	1.88		0.061	0.046	
Treatment (T)	0.34	1.00		0.48	NS		0.012	NS	
Interaction (P × T)	0.78	1.41		0.67	4.20		0.023	0.098	

* and NS indicate significance and non-significance at LSD ($P = 0.05$), respectively ($n = 10$).

Table II. Response of jamun to chemicals for percentage of seed germination and polyembryony under different propagation substrates (CP = coco-peat; SS = mixture of sand, soil and FYM in 1:1:1 ratio).

Treatment	Germination percentage			Polyembryony percentage		
	CP	SS	Mean	CP	SS	Mean
T_1 = Control	86.67 (68.85)	83.33 (66.15)	85.00 (67.50)	66.20 (55.28)*	36.11 (36.75)	51.16 (46.01)*
T_2 = EMS 0.1%	86.67 (68.85)	73.33 (59.22)	80.00 (64.04)	58.33 (50.00)	4.17 (7.53)	31.25 (28.76)
T_3 = EMS 0.5%	83.33 (66.15)	80.00 (63.93)	81.67 (65.03)	35.19 (36.06)	4.76 (8.04)	19.98 (22.05)
T_4 = Colchicine 0.1%	90.00 (74.69)	76.67 (61.22)	83.34 (67.96)	58.52 (49.50)	29.17 (27.90)	43.85 (38.95)
T_5 = Colchicine 0.5%	86.67 (71.99)	73.33 (59.71)	80.00 (65.85)	46.67 (43.07)	21.43 (27.19)	34.05 (35.14)
Mean	86.67 (70.10)	77.33 (62.05)		52.98 (46.88)	19.13 (21.49)	
For comparing the means of	S.E. ±	LSD at 5%		S.E. ±	LSD at 5%	
Propagation Substrates (P)	4.67	7.40		16.93	10.30	
Treatment (T)	0.97	NS		5.36	16.28	
Interaction (P × T)	1.87	16.54		6.90	23.02	

Figures in parenthesis pertain to the Arc sin transformation of data.
* and NS indicate significance and non-significance at LSD ($P = 0.05$), respectively ($n = 10$).

the extracted sample was pipetted out into a test tube containing 1.8 mL FRAP reagent prepared by mixing 25 mL acetate buffer (0.3 M, pH 3.6), 2.5 mL Tripyridyl-s-triazine solution and 2.5 mL $FeCl_3$ solution. The reaction mixture was kept at room temperature for 40 m and absorbance was measured at 593 nm. The standard curve was developed using different concentrations of ascorbic acid and the results were expressed as ascorbic acid equivalent antioxidant capacity.

2.4 Statistical design and analysis of data

The experiment was laid out in a factorial completely randomized design with each treatment replicated thrice. The data obtained from the experiments were analyzed using the Web Agri Stat Package version WASP2.0 (ICAR Research Complex for Goa, Ela, Goa- 403 402, India). The visual indication of data dispersion on bar and line graphs was achieved by means of the standard error of the mean. Treatment difference was evaluated using the least significant difference (LSD) at $P \leqslant 0.05$.

3 Results and discussion

3.1 Effects of the propagation substrate and chemical mutagens on germination behavior and polyembryony

Seed germination is an important parameter used to measure the response of plants to mutagenic treatments [28]. The earliness in seed germination indicates that emerged seedlings are in the active growth stage and will attain the desired height and size for grafting in a much quicker time as compared with late-emerged seedlings. We observed faster seedling emergence and completion of germination in Arka fermented coco-peat compared with the substrate containing a mixture of sand, soil and FYM (*tables I, II*). Thus, our results showed the seed germination rate, measured in terms of the germination index, in Arka fermented coco-peat to be about 37.5% higher than that of the other substrate. The effect of the propagation substrate is dependent on the nutrient as well as the aeration capacity of the substrate. Coco-peat has good physical properties,

Table III. Response of jamun to chemicals for biomass production (per plant) under different propagation substrates (CP = coco-peat; SS = mixture of sand, soil and FYM in 1:1:1 ratio).

Treatment	Shoot fresh weight (g)			Root fresh weight (g)			Shoot dry weight (g)			Root dry weight (g)		
	CP	SS	Mean	CP	SS	Mean	CP	SS	Mean	CP	SS	Mean
T_1 = Control	0.272	0.302	0.287	0.052	0.070	0.061	0.088	0.071	0.080	0.033	0.023	0.028
T_2 = EMS 0.1%	0.306	0.374	0.340	0.118	0.146*	0.132*	0.087	0.061	0.074	0.045*	0.040	0.043
T_3 = EMS 0.5%	0.351	0.427	0.389	0.116	0.109	0.113	0.070	0.090	0.080	0.033	0.034	0.034
T_4 = Colchicine 0.1%	0.346	0.446*	0.396*	0.090	0.066	0.078	0.095	0.104	0.100*	0.024	0.029	0.027
T_5 = Colchicine 0.5%	0.243	0.363	0.303	0.074	0.066	0.070	0.042	0.083	0.063	0.020	0.027	0.024
Mean	0.304	0.382		0.090	0.091		0.076	0.082		0.031	0.031	
For comparing the means of	S.E. ±	LSD at 5%		S.E. ±	LSD at 5%		S.E. ±	LSD at 5%		S.E. ±	LSD at 5%	
Propagation Substrates (P)	0.039	0.052		0.001	NS		0.003	NS		0.000	NS	
Treatment (T)	0.022	0.088		0.014	0.027		0.006	0.020		0.003	0.017	
Interaction (P × T)	0.020	0.126		0.010	0.046		0.006	0.040		0.002	0.010	

* and NS indicate significance and non-significance at LSD ($P = 0.05$), respectively ($n = 3$).

high total pore space, high water content and high cation exchange capacity, varying from 38.9 to 60.0 meq 100 g^{-1} [29], which enables it to retain large amounts of nutrients, and the adsorption complex has high contents of exchangeable K, Na, Ca and Mg [30]. Although FYM is also a source of macro- and micro-nutrients for plant growth [31], Arka fermented coco-peat might have created favorable conditions for the faster imbibition of water by seeds followed by faster germination. We found an increase in seed germination in Arka fermented coco-peat of 12% compared with the other substrate, which confirmed the findings of other authors [32, 33]. Irrespective of the propagation substrate, we found quicker seedling emergence in 0.1% colchicine (T_4)-treated seeds than the other treatments, including control (T_1). Thus, we found the stimulatory effects of low-dose colchicine treatment on seedling emergence and seed germination decreased with the increasing doses of colchicine. The results are in agreement with those of Tiwari and Mishra [34]. However, the use of different chemicals did not have any significant effect on making the germination process faster or increasing the germination percentage. Rather, we found a reduction in seed germination in chemical-treated seeds over control, which might be attributed to the effect of mutagens on the meristematic tissues of the seeds [35, 36]. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at a molecular level [37] or due to disturbance in the formation of enzymes involved in the germination process caused by EMS and colchicine [12].

The emergence of more than one seedling from a single seed due to polyembryony has already been reported in jamun [38]. However, this is the first report where a significant effect of the propagation substrate was found on emergence of multiple seedlings from a single seed (*table II*). The Arka fermented coco-peat might have created a favorable microclimate for the germination of multiple embryos, resulting in emergence of multiple seedlings from a single seed. However, the application of chemical mutagens did not have any significant effect on polyembryony.

3.2 Effects of the propagation substrate and chemical mutagens on vegetative parameters

We did not observe any significant effect of the propagation substrate on biomass production except shoot fresh weight, which was higher in the substrate containing the soil, sand and FYM mixture than Arka fermented coco-peat (*table III*). Sharma *et al.* [39] stated that the better performance of FYM may be attributed to its ability to improve the biological properties of the soil. On the other hand, sand may be responsible for producing sufficient aeration. Hence, mixing soil, sand and FYM might have helped in giving a better grip for the roots, ample aeration and sufficient organic matter, thereby increasing the shoot fresh weight. Regardless of the substrate, T_2 and T_3 produced seedlings of significantly higher shoot and root fw, as compared with T_1, which was statistically on par with seedlings obtained from T_4-treated seeds for shoot fw. The shoot dry weight was increased by 25% in T_4 as compared with T_1.

Our investigation did not find any significant effect of the propagation substrate on plant height, number of leaves or root length. Though seedling vigor was higher in Arka fermented coco-peat, it was not statistically significant. However, we found a significant improvement in the above vegetative parameters except seedling vigor in the case of seedlings raised from T_3-or T_4-treated seeds. In comparison, T_1 was statistically on par with T_2 for plant height and the number of leaves (*figure 1*). The increased values of vegetative parameters due to the lower dose of colchicine might be due to the stimulatory influence of this compound on plant morphogenesis [40]. Martin [41] reported that small doses of colchicine enhanced the action of auxin (indole-3-acetic acid) because the cells divided more actively; instead, at higher doses, colchicine led to C-mitoses and inhibited cell multiplication in *Helianthus tuberosus*. Thus, the cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume, and express more proteins with the presence of more genes. This increase in size may translate to an increase in the plant and its organs [42]. The improvement in

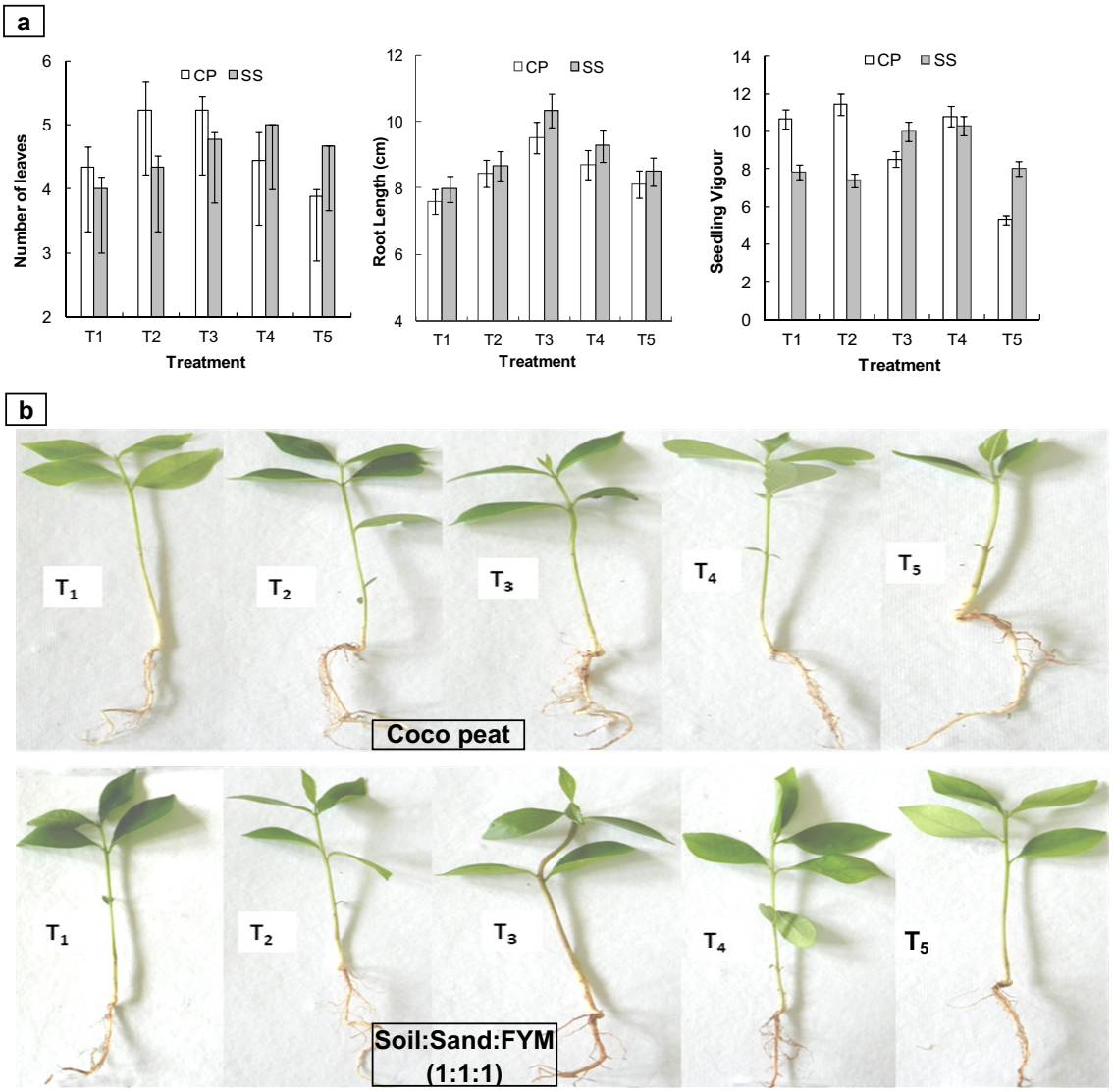

Figure 1. (a) Response of jamun to chemicals for number of leaves per plant, root length and seedling vigor under different propagation substrates (CP = coco-peat; SS = mixture of sand, soil and FYM in 1:1:1 ratio). (b) Photographs indicate plant performance at 30 days after sowing under different propagation substrates. Treatments: T_1 = control, T_2 = EMS 0.1%, T_3 = EMS 0.5%, T_4 = colchicine 0.1% and T_5 = 0.5% colchicine (EMS: ethyl methane sulfonate).

aboveground biomass in colchicine-treated plants over control might be due to increased leaf size and number of leaves per plant [43]. The production of elongated roots in colchicine-treated plants might be due to the increased auxin level, as compared with control [9]. The improvement in shoot and root weight in EMS-treated plants was also reported in papaya [10] and mulberry [44], which could be attributed to increased cell division as well as activation of growth hormones such as auxin [45].

3.3 Effects of the propagation substrate and chemical mutagens on chlorophyll content

The health status of a plant can be determined by measuring the physiological activity of that plant, indicated by the photosynthetic rate, which is related to leaf chlorophyll content. Chlorophyll, the green pigments in leaves, is very important in plant life through the process of photosynthesis. We observed 46.32, 44.44 and 46.15% higher contents of chlorophyll 'a', 'b' and total chlorophyll, respectively, in leaves of seedlings raised in Arka fermented coco-peat substrate (*table IV*). This might be due to the improved physiological condition of the seedlings grown in Arka fermented coco-peat, as humic substances in coco-peat are known to have hormone-like activity [19]. Nazari *et al.* [46] also reported significantly higher chlorophyll content in *Hyacinthus orientalis* seedlings raised in coco-peat substrate. Regardless of the substrate, we recorded significantly higher leaf chlorophyll content in seedlings obtained from T_2-, T_3- and T_4-treated seeds over T_1, which was statistically on par with seedlings obtained from T_5-treated seeds for chlorophyll 'b' content. Our findings

Table IV. Response of jamun to chemicals for chlorophyll content (in mg g^{-1} fw) under different propagation substrates (CP = coco-peat; SS = mixture of sand, soil and FYM in 1:1:1 ratio).

Treatment	Chlorophyll a			Chlorophyll b			Total Chlorophyll		
	CP	SS	Mean	CP	SS	Mean	CP	SS	Mean
T_1 = Control	1.04	0.60	0.82	0.39	0.31	0.35	1.43	0.91	1.17
T_2 = EMS 0.1%	1.66*	0.89	1.28	0.60*	0.31	0.46	2.25*	1.21	1.73
T_3 = EMS 0.5%	1.51	1.02	1.26	0.55	0.38	0.46	2.05	1.39	1.72
T_4 = Colchicine 0.1%	1.44	1.20	1.32*	0.54	0.43	0.48*	1.98	1.63	1.81*
T_5 = Colchicine 0.5%	1.31	1.01	1.16	0.50	0.37	0.43	1.81	1.38	1.59
Mean	1.39	0.95		0.52	0.36		1.90	1.30	
For comparing the means of	S.E. ±	LSD at 5%		S.E. ±	LSD at 5%		S.E. ±	LSD at 5%	
Propagation Substrates (P)	0.22	0.07		0.08	0.03		0.30	0.10	
Treatment (T)	0.09	0.10		0.02	0.06		0.11	0.14	
Interaction (P × T)	0.10	0.15		0.03	0.07		0.13	0.21	

* Indicates significance at LSD ($P = 0.05$) ($n = 3$).

Table V. Response of jamun to chemicals for biochemical content under different propagation substrates (CP = coco-peat; SS = mixture of sand, soil and FYM in 1:1:1 ratio).

Treatment	Total carbohydrates (% equivalent D-glucose)			Total phenols (GA equivalents 100 g^{-1} fw)			Total antioxidants (ascorbic acid equivalent antioxidant capacity g^{-1} fw)		
	CP	SS	Mean	CP	SS	Mean	CP	SS	Mean
T_1 = Control	33.195	24.415	28.805	18.62	25.79	22.21	3.85	4.92	4.39
T_2 = EMS 0.1%	41.000	28.317	34.659	23.14	30.42	26.78	4.77	5.94	5.36
T_3 = EMS 0.5%	30.512	27.098	28.805	28.93	32.09	30.51	6.52	6.67	6.60
T_4 = Colchicine 0.1%	50.512*	38.805	44.659	32.08	33.23	32.66	7.27	8.37	7.82
T_5 = Colchicine 0.5%	50.268	39.780	45.024*	30.34	30.62	30.48	6.75	7.47	7.11
Mean	41.098	31.683		26.62	30.43		5.83	6.67	
For comparing the means of	S.E. ±	LSD at 5%		S.E. ±	LSD at 5%		S.E. ±	LSD at 5%	
Propagation Substrates (P)	4.707	0.002		1.90	0.03		0.42	0.01	
Treatment (T)	3.612	0.006		1.84	0.05		0.62	0.01	
Interaction (P × T)	2.926	0.001		1.47	0.09		0.44	0.03	

* Indicates significance at LSD ($P = 0.05$) ($n = 5$).

were in agreement with the results of Zahedi *et al.* [47]. The amount of chlorophyll produced per gram leaf tissue is also affected by environmental conditions and the genetic composition of the plant [48]. Since T_4 is known to increase the total DNA content in plant cells [49], it might have enhanced synthesis of photosynthetic pigments as well.

3.4 Effects of the propagation substrate and chemical mutagens on total carbohydrate content

Photosynthetic activity and carbohydrates, which are photo-assimilate substances, are very important parameters to explain the physiological activity of the treated plants. Our investigation revealed that the total carbohydrate content in jamun leaves was increased by 29.72% under Arka fermented coco-peat substrate (*table V*). The increase in carbohydrate content in jamun leaves might be due to the improved photosynthetic rate of seedlings under Arka fermented coco-peat substrate [46], which might be due to its valuable characteristics including total pore space, water-holding capacity and cation exchange capacity. Irrespective of the substrate, we recorded 55.04 and 56.31% increase, respectively, in the total carbohydrate content of leaves in T_3 and T_4 over T_1. The increased leaf size and photosynthetic pigments due to colchicine treatments might have increased the total carbohydrate production in leaves. Warner and Gerald [50] suggested that the photosynthetic rate per unit leaf area is related to the number of photosynthetic cells per unit area. As a matter of fact, the photosynthetic rate per cell is correlated with the amount of DNA per cell, which is increased in colchicine-treated plants [49].

3.5 Effects of the propagation substrate and chemical mutagens on total phenolics

Phenolics are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. They are known to be antimicrobial and activators of

plant defense genes [51]. Our investigation showed a 14.31% higher content of total phenolics in jamun leaves under the substrate containing the mixture of sand, soil and FYM than Arka fermented coco-peat (*table V*). This might be due to the better aeration and water retention capacity of Arka fermented coco-peat substrate. Saikia and Upadhyaya [52] suggested that the production of secondary metabolites such as phenolics in plants is related to their growing conditions. As oxidative signaling controls both synthesis and accumulation of secondary metabolites in plants [53], thereby, it may be hypothesized that plant-growing in stress conditions would generate higher concentrations of phytochemicals resulting from the diversion of carbon skeletons from protein synthesis [54]. The seedlings obtained from T_4-treated seeds had 47.07% higher total phenolic contents, as compared with T_1, which might be related to a plant protective mechanism against stress. Changing the metabolic profile in colchicine-treated plants can be interpreted as the cause of an alteration in the mechanism(s) which regulates the biosynthesis of several compounds [55]. As colchicine affects the cellular DNA level [49], it also affects protein metabolism and thus alters the plant metabolism, leading to the generation of reactive oxygen species. Plants protect themselves from this harmful radiation by synthesizing phenolic compounds, which act as a screen inside the epidermal cell layer, and by adjusting the antioxidant systems at both cell and whole organism levels [56].

3.6 Effects of the propagation substrate and chemical mutagens on total antioxidants

The present study showed the significant influence of the substrate containing a mixture of sand, soil and FYM on jamun seedlings, stimulating 14.41% higher production of total antioxidants over Arka fermented coco-peat substrate (*table V*), which might be associated with the enhanced phenolic content in seedlings grown in the above substrate [57]. Irrespective of the propagation substrate, we observed 78.54% higher accumulation of total antioxidants in leaf cytoplasm in T_4 over T_1, which might be due to higher accumulation of phenolic compounds in colchicine-treated plants. Thus, our results suggested that the phenolic content of plants contributes directly to their antioxidant action [58].

4 Conclusion

Of the different doses of chemicals used in our study, colchicine at 0.1% had significant effects on initiation of seed germination, shoot biomass production, seedling height, number of leaves, root length and leaf biochemical compounds such as chlorophyll and carbohydrates. Arka fermented coco-peat as a propagation substrate was significantly superior to the mixture of sand, soil and FYM in terms of vegetative growth parameters such as seed germination and the rate of seedling emergence, polyembryony, production of chlorophyll pigments and total carbohydrates in leaves, and in terms of accumulation of stress-related metabolites such as total phenolics and total antioxidants. As superior healthy rootstock is necessary for high grafting success under nursery conditions, seeds of jamun treated with 0.1% colchicine can produce healthy vigorous seedlings grown on Arka fermented coco-peat substrate.

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