AATCC Review

07 July 2025: Received
26 August 2025: Revised
04 September 2025: Accepted
02 October 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article | Open Access

Induction and assessment of colchicine induced variability in karonda

Krishna[1], V. P. Singh*[1], S. K. Verma[2], Ajay Kumar[3], Ratna Rai[1], Rajesh Kumar[1], Satish Chand[1], Viveka Nand[4] and Virendra Kumar[5]

[1]Department of Horticulture, GB Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, 263145, India
[2]Department of Genetics and Plant Breeding, GB Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, 263145, India
[3]Department of Agronomy, GB Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, 263145, India
[4]Department of Chemistry, GB Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, 263145, India
[5]School of Agriculture & Development Studies, Uttarakhand Open University, Haldwani, Uttarakhand (India) , India

ABSTRACT

Karonda (Carissa carandas L.) is a native fruit known for its nutrition, medicinal benefits and diversified uses. Despite its multiple uses, it exhibits a narrow genetic base. Since genetic variability is a prerequisite for any effective crop improvement programme, the present study was conducted to induce variation in this crop. Seeds of Karonda cv. Pant Manohar was treated with different doses of colchicine, ranging from 0.1%-0.9% along with a control. The experiment was conducted using a randomised block design (RBD), with each treatment replicated thrice. The main objective of the current study was to assess the ability of colchicine to induce variation in karonda. Plants showing some variations were selected for further biochemical analysis to check the effectiveness of colchicine in creating variation. Variants were evaluated against the control ones for leaf chlorophyll content, total carotenoid content and relative water content (RWC) in the leaves. The results showed dose-independent variations among the treatments. Whereas, C_5 (0.5%) exhibited maximum chlorophyll and carotenoid content, while maximum RWC was recorded in C_2 (0.2%). Morphological variations were also observed in treated population. These findings may be further utilized in future crop improvement programmes aimed at variability creation, and genetic improvement in karonda.

Keywords: *Karonda, colchicine, chlorophyll, carotenoid, variation, correlation, crop improvement.*

Introduction

The increasing demand for nutritionally rich and therapeutic fruits highlights the significance of underutilised and minor fruit crops. Their nutrient-dense composition, resilience to biotic and abiotic stresses and potential to alleviate hidden hunger or deficiencies of different vitamins make these fruits vital for sustainable production. With increasing awareness of health and nutrition, underutilised fruit crops with medicinal value emerge as vital resources for developing safe, affordable and functional therapeutic foods (1). One such potential fruit crop is karonda (*Carissa carandas* L.), which is a rich source of iron and vitamin C. This hardy, evergreen, spiny, native shrub is a member of the Apocynaceae family and grows in arid and semiarid climates worldwide. Its fruit has been traditionally used in the ancient Indian Ayurvedic system of medicine for treating acidity, indigestion, fresh and infected wounds, skin ailments, urinary disorders, diabetic ulcers, biliousness, stomach pain, constipation, anaemia, anorexia and even certain mental health conditions (2). Karonda is recognised as a nutrient-dense fruit, rich in iron and providing substantial quantities of vitamins C, A and B-complex, along with essential minerals such as calcium, phosphorus, potassium, sodium and sulphur (3).

*Corresponding Author: ***V. P. Singh***

DOI: https://doi.org/10.21276/AATCCReview.2025.13.04.648

It is a multipurpose crop, its mature fruits are processed into a number of products such as chutney, pickle and sauces, while ripe fruits are used for jam, jelly, beverages and candy (4), whereas its plants are commonly cultivated in home gardens for hedging or ornamental purposes, especially in tropical and subtropical regions.

Despite its multiple uses, karonda improvement has largely relied on selection from naturally existing genetic variation in the germplasm. Traditional breeding methods are often time-consuming and labour-intensive. Colchicine, a mitotic inhibitor and potent chemical agent, is known to induce polyploidy and enhance genetic variability in various crops within a short period (5). It functions as a chromosome-doubling agent and can also induce mutations, with mutants generated using colchicine commonly referred to as "colchi-mutants" (6). The mutagenic effects of colchicine on plant performance have been documented in earlier studies (7, 8, 9). Variability induction and ploidy manipulation through physical and chemical agents may provide a powerful tool to generate populations with novel traits, which can then be effectively characterised and utilised (10). The useful traits usually emerge from changes in plant structure or physiology, which play an important role in crop improvement (11). Hence, the present study aimed to create genetic variation in karonda using colchicine treatment and to assess its impact on various biochemical traits.

Material and Methods

Experimental site and treatment details: The present study was conducted at Medicinal Plants Research and Development

Centre, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India and seeds of 'Pant Manohar' karonda were used for colchicine treatment. Seeds were treated with 9 concentrations of colchicine, *viz*. 0.10% to 0.90% along with control. Colchicine solutions were prepared in 1% dimethyl sulfoxide (DMSO) to improve permeability and penetration of colchicine into cells (12). Seeds were soaked for 24 hours in colchicine solution, and for control, seeds were soaked in distilled water. Treated seeds were then rinsed thoroughly and sown in pro-trays having a media mixture of sand: soil: vermicompost (1:1:2).

Biochemical analysis: Among the treatment plants showing variation were selected, and then their biochemical analysis was done. Chlorophyll a, b, total chlorophyll and total carotenoid contents were estimated in fully expanded fresh leaf samples and were determined according to Hiscox and Israelstam (13).

Chl a (mg/g FW) = (12.7 x A663 − 2.69 x A645) V / 1000 x W
Chl b (mg/g FW) = (22.9 x A645 – 4.48 x A663) V / 1000 x W
Total Chl (mg/g FW) = (20.2 x A645 + 8.02 x A663) V / 1000 x W
Total carotenoids (mg/g FW) = (A480 + 0.11 x A663– 0.638 x A645) V / 1000 x W

where, A = absorbance of chlorophyll extract at specific wavelength, V = final volume of the sample; W = weight of tissue extracted on fresh weight basis.
The relative leaf water content was estimated using the formula of Turner (14):

$$RLWC\ (\%) = \frac{Fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight} \times 100$$

Statistical analysis:
The experiment was conducted in a Randomized Block Design (RBD) with three replications. Experimental data were analyzed through the standard analysis of variance (ANOVA) procedure using the RStudio software (4.5.1), followed by the least significant difference (LSD) test. Statistical significance was determined at $p \leq 0.05$.3.

Results and Discussion

3.1. Effect of colchicine on morphological parameters

Various colchicine treatments significantly influenced plant height, number of leaves, and number of branches in karonda (Table 1). Plant height exhibited significant variation ($LSD_{0.05}$ = 2.84) among treatments, ranging from 8.10 cm at 0.40% colchicine to 13.83 cm at 0.30% colchicine. The highest mean plant height was observed at 0.30%, which was statistically at par with the control, C1, C5, and C6. However, a reduction in height was recorded at C4 (8.10 cm) and C2 (10.58 cm), and complete growth inhibition occurred at 0.90% colchicine, indicating a strong cytotoxic effect at higher doses. Reduction in plant height may be attributed to the antimitotic nature of colchicine. It binds to the cell protein tubulin and arrests cell division at the metaphase stage by preventing spindle formation, leading to the breakdown of microtubules in cells, reducing cell movement, metabolism, and activity. As a result, plant growth and development slow down because of disturbed physiological processes and a lower rate of cell division (15). Amiri et al. (16) observed that colchicine-treated plants showed slower growth, altered morphology, and delayed flowering in polyploids. These effects may be linked to reduced mitotic and cell division rates in larger cells containing more chromosomes. The total plant height was lower in tetraploid lines than in diploids, mainly due to shorter internodes. Similar reductions in plant height after colchicine treatment were also reported by (17) in ber, (18) in chrysanthemum, (19) in radish, and (20) in gladiolus.Similarly, the number of leaves and branches exhibited significant variation among the different colchicine concentrations (Table 1). The number of leaves ranged from 29.67 to 51.50, while the number of branches varied from 2.33 to 5.67. The maximum number of leaves per plant (51.50) was recorded in control (C0), followed by C6 (51.33), whereas the maximum number of branches per plant (5.67) was recorded in plants treated with C6 and C3, followed by control (5.00). The lowest values for both traits (29.67 leaves and 2.33 branches) were observed at C4, indicating a mild inhibitory effect on vegetative growth at this concentration. The reduced growth may be attributed to increased activity of growth inhibitors, a decrease in auxin levels, or the presence of substances that inhibit auxin synthesis, as reported by (21) in carnation and (22) in silver cock's comb.

3.2. Effect of colchicine on biochemical parameters

The effect of colchicine treatment on different biochemical parameters of karonda seedlings is presented in Table 1. The content of chlorophyll a varied in the range of 2.04–2.63 mg/g FW, with the maximum concentration in the C4 treatment. Chlorophyll b content ranged from 0.47 to 0.77 mg/g FW, with the highest value recorded at C5. Total chlorophyll content significantly improved at 0.50% colchicine (3.35 mg/g FW) compared to other treatments. Total carotenoid content reached a maximum at 0.50% colchicine (0.16 mg/g FW) and thereafter decreased to 0.11 mg/g FW at 0.80% colchicine. Relative water content (RWC) showed fluctuations but remained relatively high at 0.20%, 0.50%, 0.70%, and 0.80% colchicine treatments. The increase in chlorophyll and carotenoid contents at moderate colchicine doses (0.50%) suggests enhanced synthesis or retention of photosynthetic pigments. Similarly, (23) reported improved chlorophyll levels in colchicine-treated guava seedlings and suggested that colchicine may improve photosynthetic activity through polyploidy. (24) also reported that ginger plants treated with various colchicine concentrations exhibited increased pigment levels up to an optimum, followed by a decline at toxic concentrations. RWC varied in response to colchicine treatments and was highest at C2 (0.20%), followed by C8 (0.80%), with no significant difference between their values, suggesting improved passive water retention, cellular turgor, and water balance in colchicine-induced polyploid plants (25). In contrast, decreases in RWC at some doses may indicate physiological stress in seedlings, as reported by (26) in cowpea, faba bean, and soybean. At higher colchicine doses (0.60% and above), reductions in pigment content and water status indicate harmful stress that outweighs beneficial effects. This biphasic response aligns with typical colchicine studies, where low to moderate doses enhance biochemical activity while higher doses cause cellular damage. In the present study, a moderate colchicine dose, C5 (0.50%), resulted in the highest levels of photosynthetic pigments and water content in karonda, suggesting it as the optimal concentration to harness polyploidy benefits while minimizing adverse effects, as previously reported in guava, turmeric, and sweet potato (24, 23, and 27).

Table 1: Effect of colchicine treatment on biochemical attributes

Colchicine concentration (%)	Plant height (cm)	No. of leaves per plant	No. of branches per plant	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Total Carotenoid (mg/g FW)	Relative water content (%)
C_0 (0.00)	13.55^{a}	51.50^{a}	5.00^{a}	2.06^{e}	0.49^{f}	2.50^{e}	0.12^{bcd}	78.30^{ab}
C_1 (0.10)	13.43^{a}	46.33^{a}	4.67^{a}	2.29^{d}	0.60^{d}	2.89^{c}	0.12^{bcd}	66.87^{b}
C_2 (0.20)	10.58^{bc}	44.67^{a}	4.17^{ab}	2.31^{cd}	0.62^{d}	2.90^{c}	0.14^{abc}	86.15^{a}
C_3 (0.30)	13.83^{a}	44.67^{a}	5.67^{a}	2.47^{bc}	0.66^{c}	3.12^{b}	0.14^{ab}	76.54^{ab}
C_4 (0.40)	8.10^{c}	29.67^{b}	2.33^{b}	2.63^{a}	0.70^{b}	3.26^{a}	0.13^{bcd}	66.63^{b}
C_5 (0.50)	13.05^{ab}	49.50^{a}	4.67^{a}	2.53^{ab}	0.77^{a}	3.35^{a}	0.16^{a}	75.69^{ab}
C_6 (0.60)	12.90^{ab}	51.33^{a}	5.67^{a}	2.25^{d}	0.54^{e}	2.74^{d}	0.12^{cd}	67.37^{b}
C_7 (0.70)	12.10^{ab}	48.33^{a}	3.67^{ab}	2.16^{de}	0.61^{d}	2.69^{d}	0.13^{bcd}	78.25^{ab}
C_8 (0.80)	11.57^{ab}	45.00^{a}	4.00^{ab}	2.04^{e}	0.47^{e}	2.47^{e}	0.11^{d}	84.06^{a}
C_9 (0.90)	0.00^{d}	0.00^{c}	0.00^{c}	0.00^{f}	0.00^{g}	0.00^{f}	0.00^{e}	0.00^{e}
LSD (P≤0.05)	2.84	14.92	2.21	0.16	0.03	0.12	0.02	14.61

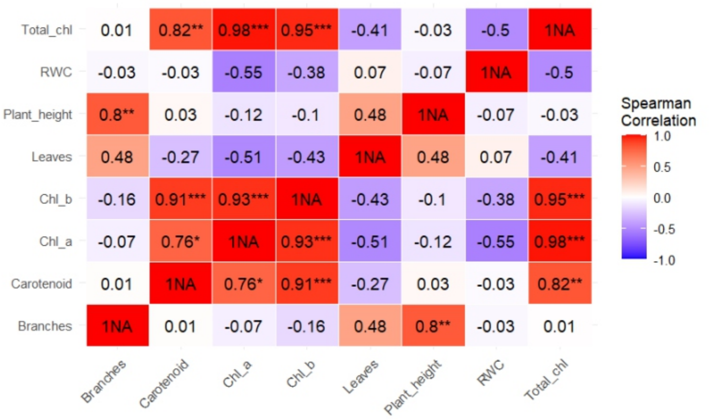

Fig 1: Heatmap showing correlation between physical and biochemical parameters of karonda

3.3 Correlation matrix of morphological traits, photosynthetic pigments and relative water content in karonda:

The correlation matrix (Table 2) revealed significant interrelationships among the traits studied. Total chlorophyll content showed a strong positive correlation with chlorophyll a (r = 0.98), chlorophyll b (r = 0.95) and carotenoid content (r = 0.82), indicating a close association between the photosynthetic pigments. A highly significant correlation was also observed between chlorophyll a and b (r = 0.93), indicating their coordinated accumulation within the photosynthetic system. Plant height showed a positive relationship with the number of branches (r = 0.80), suggesting that increased plant stature was accompanied by enhanced branching. In contrast, relative water content (RWC) exhibited weak or negative correlations with most traits, suggesting that leaf water status was largely independent of both growth and pigment characteristics.

Table 2: Correlation matrix of photosynthetic pigments and relative water content in karonda

Traits	Branches	Carotenoid	Chl a	Chl b	Leaves	Plant height	RWC	Total chlorophyll
Branches	1.00	0.01	-0.07	-0.16	0.48	0.80**	-0.03	0.01
Carotenoid		1.00	0.76*	0.91***	-0.27	0.03	-0.03	0.82**
Chl a			1.00	0.93***	-0.51	-0.12	-0.55	0.98***
Chl b				1.00	-0.43	-0.10	-0.38	0.95***
Leaves					1.00	0.48	0.07	-0.41
Plant height						1.00	-0.07	-0.03
RWC							1.00	-0.50
Total chl								1.00

***$p < 0.05$, **$p < 0.01$, ***$p < 0.001$*

Conclusion

It can be concluded that colchicine treatment effectively induced variations in karonda indicating its potential application in karonda crop improvement programmes. The observed variations, though not directly related to colchicine concentration, exhibited both stimulatory and inhibitory effects on the plant's morphological and biochemical traits. Correlation matrix suggested that strong positive correlations were observed among photosynthetic pigments, whereas relative water content showed a weaker and more complex correlation pattern, suggesting that leaf water status was largely independent of both growth and pigment characteristics. Further, it is crucial to conduct a thorough assessment of the mutant population across diverse traits to identify promising variants for future breeding and crop improvement programs.

Future scope of study

Future research can focus on enhancing the ploidy level of the plants as polyploid plants frequently have superior characteristics such as larger fruit size, higher production, disease resistance, and tolerance to environmental challenges such as high temperatures and drought. Triploidy, a desirable feature in fruit crops, results in seedlessness and increased vigour. These plants can be used as parent for further future breeding programme for crop improvement of karonda. Breeders can address customer demand for larger, seedless fruits without resorting to direct genetic manipulation by inducing polyploidy with colchicine. Since mutation breeding is the easiest method to create genetic variation in plants within a short duration, therefore colchicine can be used as a potent mutagen to induce variations.

Acknowledgement

The authors are thankful to the Head, Department of Horticulture and Director Research, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India) for providing necessary field and laboratory facilities for conducting the research trial.

Declaration: No potential conflict of interest was reported by the authors.

References

1. Singh, V. P., Krishna, Mishra, D.S., Yadav, V., Rai, R., Kumar, V., Singh, O., Kumar, R., Sane, R., and Ravat. P. 2025 Karonda (*Carissa carandas* L.): A review on its varietal wealth, production and diversified uses in India. Plant Science Today (accepted). https://doi.org/10.14719/pst.10226

2. Jadhav, S. D., Nangare, D. D. and Kakade, V. S. (2022). *Karonda: Under-exploited fruit crop for dry land condition.* Just Agriculture.

3. Nisha, K., Murugesan, B., Alaguthevar, R., Thirupathi, P., Iyyamperumal, M. and Rajagounder R. Moisture-dependent physicochemical characteristics and microstructure of karonda (*Carissa carandas* L.) fruit. *Plant Sci. Today*.12(2).

4. Dalal, R.P.S., Thakur, N.A. and Singh, A., 2010. Nutritional value of karonda (Carissa *carandas* Linn.) – A non-conventional fruit under semi-arid condition of Punjab. Indian J. Agroforest., 12 (2):102–104.

5. Singh, B., Yun, S., Gil, Y., and Park, M. H. (2025). The Role of Colchicine in Plant Breeding. *International Journal of Molecular Sciences*, 26(14), 6743.

6. Ari E, H Djapo, N Mutlu, E Gurbuz and O Karaguzel (2015). Creation of variation through gamma irradiation and polyploidization in *Vitex agnuscastus* L. *Sci. Hortic.* 195: 74-81.

7. Balkanjieva J (1980) Influence of genotype on mutagenic variability in barley (*H. vulgare* L.) following colchicine treatment. *Barley Genet. Newsl.* 10: 7-10.

8. Castro CM, AD Oliveira and FD Carvalho (2003) Changes in allele frequencies in colchicine-treated ryegrass populations assessed with RAPD markers. *Braz. J. of Sci.* 9: 107-112.

9. Singh K, O.P. Awasthi, A. Singh, P. Yadav and S. Kumar (2025). Colchicine Induced Variation in Sweet Orange (*Citrus sinensis* (L.) Osbeck) cv. Mosambi. *Indian J. Plant Genet. Resour*. 38(2), 192-197. DOI: 10.61949/0976-1926.2025.v38i02.08

10. Grosser, J.W., Kainth, D. and Dutt, M. (2014). Production of colchicine-induced autotetraploids in pummelo (*Citrus grandis* Osbeck) through indirect organogenesis. *Hortic. Sci.* 49: 944-948.

11. Mallick, M., Awasthi, O.P., Paul, V., Verma, M.K. and Jha, G. (2016) Effect of physical and chemical mutagens on leaf sclerophylly and stomatal characteristics of Kinnow mandarin mutants. *Indian J. Hortic.* 73: 291-293.

12. Yang, X.M., Cao, Z.Y., An, L.Z., Wang, Y.M. and Fang, X.W. 2006. *In vitro* tetraploid induction via colchicine treatment from diploid somatic embryos in grapevine (*Vitis vinifera* L.). Euphytica 152, 217–224. https://doi.org/10.1007/s10681-006-9203-7

13. Hiscox, J. D. and Israelstam, G. F. 1979. A method for the extraction of chlorophyll from the leaf tissue without maceration. *Can. J. Bot.*, 57: 1332-1334.

14. Turner, N. C. 1981. Techniques and experimental approaches for the measurement of plant water status. *Plant & Soil*, 58: 339-366.

15. Atichart, P. (2013). Polyploid induction by colchicine treatments and plant regeneration of *Dendrobium chrysotoxum*, *Thai Journal of Agricultural Science*. 46(1): pp. 59-63.

16. Amiri, S., Kazemitabaar, S.K., Ranjbar, G. and Azadbakht, M. (2010). The effect of trifluralin and colchicine treatments on morphological characteristics of Jimsonweed *(Datura stramonium L.). Trakia Journal of Sciences*. 8(4)*:*47-61.

17. Gu, X. F., Yang, A. F., Meng, H., and Zhang, J. R. (2005). In vitro induction of tetraploid plants from diploid Zizyphus jujuba Mill. cv. Zhanhua. *Plant cell reports*, *24*(11), 671–676. https://doi.org/10.1007/s00299-005-0017-1

18. Kushwah, K. S., Verma, R. C., Patel, S. and Jain, N. K. (2018). Colchicine induced polyploidy in Chrysanthemum carinatum L. *Journal of Phylogenetics & Evolutionary Biology*, 6(1), 2.

19. Manawadu, I. P., N. Dahanayake and S. G. J. N. Senanayake. 2016. Colchicine induced tetraploids of radish (*Raphanus sativus* L.). Trop. Agric. Res. Ext. 19:176-179.

20. Manzoor, A., Ahmad, T., Bashir, M. A., Baig, M. M. Q., Quresh, A. A., Shah, M. K. N., & Hafiz, I. A. (2018). Induction and identification of colchicine induced polyploidy in *Gladiolus grandiflorus* 'White Prosperity'. *Folia horticulturae*, 30(2), 307-319.

21. Roychowdhury R, Tah J. Chemical Mutagenic Action on Seed Germination and Related Agro-metrical Traits in M1 *Dianthus* generation. *Curr Bot*. 2011; 2:19-23.

22. Mostafa GG, Alfrmawy AM and El-Mokadem HE. Induction of mutations in *Celosia argentea* using dimethyl sulphate and identiication of genetic variation by ISSR markers. *Afr J Biotechnol*. 2014; 13:106-111.

23. Mulagund, J., Vishwakarma, P. K., Dinesh, M. R., Vasugi, C., Rekha, A. and Ravishankar, K. V. (2023). In situ induction of polyploidy in guava (*Psidium guajava* L.). *Erwerbs-Obstbau*, 65(6), 2479-2490.

24. Prasath, D., Nair, R. R., and Babu, P. A. (2022). Effect of colchicine induced tetraploids of ginger (*Zingiber officinale* Roscoe) on cytology, rhizome morphology and essential oil content. *Journal of Applied Research on Medicinal and Aromatic Plants*, 31, 100422.

25. Ntuli, N. R., and Zobolo, A. M. (2008). Effect of water stress on growth of colchicine induced polyploid *Coccinia palmata* and *Lagenaria sphaerica* plants. *African Journal of Biotechnology*, 7(20).

26. Mangena, P., and Mushadu, P. N. (2023). Colchicine-induced polyploidy in leguminous crops enhances morpho-physiological characteristics for drought stress tolerance. Life, 13(10), 1966.

27. Zhang, X., Tang, C., Jiang, B., Zhang, R., Li, M., Wu, Y. and Wang, Z. (2025). Refining polyploid breeding in sweet potato through allele dosage enhancement. *Nature Plants*, 11(1), 36-48.