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COMPARATIVE MORPHOLOGICAL ANALYSIS OF TOMATO SOMACLONES IN FIELD CONDITIONS

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Abstract

The instability of *in vitro* cultures may cause genetic and epigenetic changes in crops called somaclonal variations. Sometimes, these changes produce beneficial effects, which can be used in breeding programs to generate new cultivars with desirable characteristics. To find out the presence of beneficial variations among *in vitro* regenerated plants, the current study was carried out at the Horticulture Division, Bangladesh Institute of Nuclear Agriculture (BINA). Seven somaclones from two different donor parents were evaluated in this study. The result revealed significant phenotypic diversity among the somaclones and their parents, especially in plant height, leaf morphology, flower type, fruit shape, and yield components. Findings show that, among the somaclones derived from Binatomato-11, IVRP 11.6 had the highest no. of fruits plant^{-1} (89) and yield plot^{-1} (32.16 kg), whereas IVRP 11.3 had the highest single fruit weight and the best fruit quality with the highest total soluble solids (TSS) content (5.89 % brix). On the other hand, the highest yield plot^{-1} (39.86 kg) was produced by IVRP 13.1, which was 3.5 times higher than parent Binatomato-13, but it was not better in any other yield-related characteristics. In order to improve tomato productivity and adaptation to changing climatic conditions, somaclonal variations can be integrated into future plant breeding techniques.

Key words: Phenotype, somaclonal variation, crop improvement, genetic and epigenetic change, plant tissue culture

Introduction

Tomato (*Solanum lycopersicum*, 2n = 2x = 24), a member of the Solanaceae family, is a nutritionally rich crop valued for its content of vitamins A and C, iron, dietary fiber, and antioxidants such as lycopene, β-carotene, flavonoids, and lutein (Devi *et al*., 2008; Block *et al*., 1992; Gerster, 1997; Rao and Agarwal, 2000). Lycopene, in particular, has been linked to antioxidative and anticancer properties, contributing to the crop's rising global demand (Bhowmik *et al*., 2012; Wu *et al*., 2011; Raiola *et al*., 2014).

In Bangladesh, tomato production reached 469,204.49 tons in 2022–23, 26,904.89 tons increase from the previous year (Anonymous, 2024a). Despite this growth, $11.9 million worth of tomatoes were imported in 2023 to meet rising demand (Anonymous, 2024b). Meeting this demand under climate stress requires the development of improved cultivars with enhanced biotic and abiotic stress resistance.

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Plant tissue culture (PTC), a biotechnological tool, facilitates rapid, season-independent propagation true to type plant production and can induce novel genetic variation through somaclonal variation (Larkin and Scowcroft, 1981; Bairu *et al*., 2011; Sudheer *et al*., 2022). Nevertheless, the deviation of the concept was first observed and reported in cell and tissue cultures by Braun (1959) and defined as somaclonal variation (Larkin and Scowcroft, 1981). This genetic instability (Somaclonal variation) poses significant challenges to the propagation of clonal genotypes. Although often viewed as a challenge to clonal fidelity, somaclonal variation can generate beneficial traits for crop improvement for instance resistance to biotic and abiotic stress and high productivity can supplement the global economy. (Sudheer *et al*., 2022; Mehta and Angra, 2000; Anil *et al*., 2018, Bouharmont, 1994).

Factors such as plant growth regulators, particularly Benzylaminopurine (BAP), Indole Butyric acid and repeated subcluturing and prolonged exposure to *in vitro* conditions are key inducers of such variation. In addition, BAP doses of 1 mgL^{-1}, 2 mgL^{-1} and 3 mgL^{-1} doses depicted as preferrable for inducing somaclonal variation (Ferreira *et al*., 2023; Arun *et al*., 2007).

Somaclonal variation in different plant species can be assessed by biochemical, morphological, and molecular based markers methods (Hannachi *et al*., 2021; Patel *et al*., 2021). Key morphological indicators include plant height, leaf morphology, the formation of floral buds, the size, shape and color of the fruit and abnormal pigmentation (Israeli *et al.*, 1991). Reports on the assessment of somaclonal variation based on morphological traits in sweet cherry (*Prunus avium)* (Ali *et al*., 2018) and on qualitative and quantitative traits in tomatoes (Ghasemi *et al*., 2015) have been published.

So, the current study was conducted with a view to evaluating morphological variation among *in vitro* regenerated plants compared to their parents.

Materials and Methods

This study was conducted at the Horticulture farm, BINA, HQs, Mymensingh, during the period from December 2023 to February 2024. The experimental farm was under the old Brahmaputra floodplain soil (AEZ 29) having average temperature 75°F at November 2023 and 67°F at December 2023. R_1 seeds of the tomato somaclonal genotypes IVRP 11.3, IVRP 11.4, IVRP 11.5, IVRP 11.6, IVRP 13.1, IVRP 13.2 and IVRP 13.3 (Collected seed from selected plants of R_0 generations of two parents namely Binatomato-11, and Binatomato-13) were transplanted in the field of Horticulture farm on November 28, 2023. The experiment was carried out using Randomized Complete Block Design (RCBD) with three replications and the plot size was 2m×2m. During the growing season, fertilizer was applied according to the Fertilizer recommendation guide-2018 (140 kgha^{-1} N, 128 kgha^{-1} K, 30 kgha^{-1} S) (Ahmmed *et al.*, 2018). Notably, the former experiment was executed in MS media supplemented with 2.0 mgL^{-1} BAP+0.5 mgL^{-1} IAA using cotyledon as explant. After that, this study was extended to detect somaclonal variation of R_1 plants regenerated from

Binatomato-11 and Binatomato-13 compared to their donor parents in the winter season based on some morphological traits. The evaluation encompassed a comprehensive analysis of several parameters, including leaf color, leaf type, fruit shape, plant height, days to flowering, days to fruit set, number of branches plant^{-1}, number of clusters plant^{-1}, number of flowers cluster^{-1}, weight of individual fruit, number of fruits plot^{-1}, yield plot^{-1}, no. of fruits plant^{-1}, fruit length, fruit breadth, length-to-breadth ratio, and total soluble solids (TSS).

The data were statistically analyzed using the analysis of the variance (ANOVA), followed by Fisher's LSD test to pinpoint the significant differences. Analyses were performed using RStudio software (R Core Team, 2024).

Results and Discussion

The data (Table 1 and Fig. 1) reveals a consistent pattern in leaf morphology across the majority of examined genotypes. Regarding leaf color, all somaclonal variants derived from Binatomato-11 were similar to the parent, with the exception of IVRP 11.5, which exhibited dark green leaves (Fig. 1). In case of Binatomato-13, all *in vitro* regenerated genotypes showed variation compared to parent, producing dark green leaves as opposed to the light green of the parent. However, there was no differences among somaclonal variants and parent (Binatomato-11) in leaflet orientation, leaf type and leaf blade shape. On the other hand, IVRP 13.3 showed exceptional leaflet orientation, that was opposite, whereas, all genotypes showed alternate leaflet orientation derived from binatomato-13. Furthermore, IVRP 13.2 displayed potato-type leaves with an oval shape, while all other genotypes presented lance-shaped leaves with serrated edges, which is the characteristic of typical tomato plants (Fig. 2). Notably, intercalary leaflets were present in maximum *in vitro* regenerated genotypes, with the exception of IVRP 11.3, IVRP 11.4 and IVRP 13.3. Mentionable matter is, only leaves with five to nine nodes below the shoot apex were taken in order to compare leaves at similar developmental stages. Considering the flower shape, all somaclonal genotypes derived from Binatomato-11 and Binatomato-13 exhibited star shaped flowers. The exception was IVRP 13.3, which displayed marigold type flowers (Table 1, Fig. 1 and Fig. 2).

The majority of genotypes produce red fruits in a variety of shapes, including round, ovate, and flattened, demonstrating the diversity of fruit colors and shapes. The lines of IVRP 11.3 and IVRP 11.4 generated fruits with an ovate shape and red coloration, whereas IVRP 11.5 produced flattened fruits, and IVRP 11.6 yielded round-shaped fruits (Table1 and Fig. 1). These results indicate variation from the parent, Binatomato-11, which typically produced ellipsoid-shaped fruits. In contrast, all the *in vitro* regenerated somaclones derived from Binatomato-13 displayed flattened fruit morphology, despite the parent cultivar typically bearing round-shaped fruits (Fig. 2).

Producing red colored fruit was a uniform trait among all genotypes, whereas, a diversity of fruit shapes was observed across the genotypes. It is known that, the color of the fruit is determined by the quantities of yellow to red carotenoids, such as lycopene and β-carotene, the green chlorophyll, the yellow flavonoid naringenin chalcone, and purple anthocyanins and lycopene is the primary pigment responsible for the red color of tomatoes (Roohanitaziani, 2019). Therefore, the red fruit color represents the possibility of abundant lycopene presence. On the other hand, the range of shapes in all genotypes included round, ovate, and flattened shapes. This diversity may be essential for the further varietal development through induced mutation or hybridization, considering marketability and customer preference.

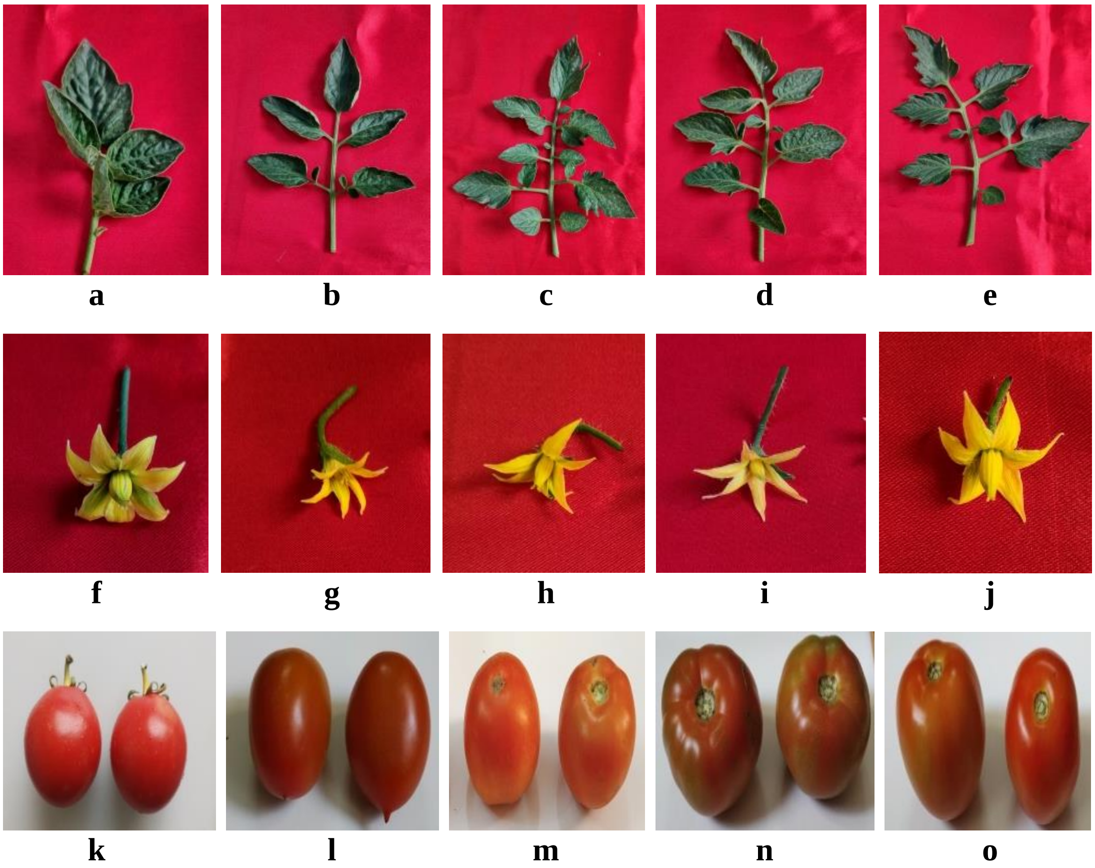

Fig. 1. Diversity in leaf, flower and fruit shapes in different somaclonal varients of Binatomato-11 compared to parent (a. Leaf of IVRP 11.3, b. Leaf of IVRP 11.4, c. Leaf of IVRP 11.5., d. Leaf of IVRP 11.6, e. Leaf of Binatomato-11, f. Flower of IVRP 11.3, g. Flower of IVRP 11.4, h. Flower of IVRP 11.5, i. Flower of IVRP 11.6, j. Flower of Binatomato-11, k. Fruits of IVRP 11.3, l. Fruits of IVRP 11.4, m. Fruits of IVRP 11.5, n. Fruits of IVRP 11.5, o. Fruits of IVRP 11.6, p. Fruits of Binatomato-11.

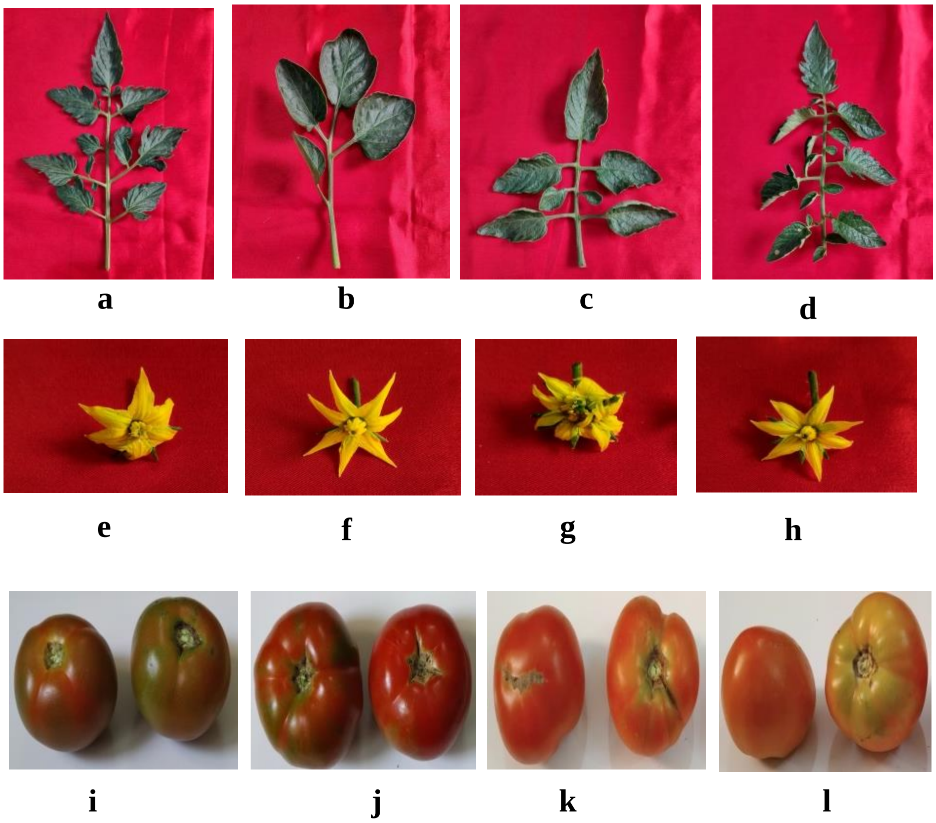

Fig. 2. Diversity in leaf, flower and fruit shapes in different somaclonal varients compared to parent (a. Leaf of IVRP 13.1, b. Leaf of IVRP 13.2, c. Leaf of IVRP 13.3, d. Leaf of Binatomato-13, e. Flower of IVRP 13.1, f. Flower of IVRP 13.2, g. Flower of IVRP 13.3, h. Flower of Binatomato-13, i. Fruits of IVRP 13.1, j. Fruits of IVRP 13.2, k. Fruits of IVRP 13.3, l. Fruits of Binatomato-13)

The results presented in Fig. 3 and Fig. 4 indicated that IVRP 11.6 was the tallest genotype having the highest number of branches (89.57 cm; 6.50), while the shortest one with the lowest branches was IVRP 11.4 (54.71 cm; 1.50) compared to parent and all other somaclonal variants derived from Binatomato-11. In terms of flowering, IVRP 11.6 took the longest time to flower, requiring 39 days, whereas IVRP 11.3 flowered the earliest at 31 days. However, the maximum time that was 24.67 was required to fruit set in IVRP 11.3, while IVRP 11.4 and IVRP 11.5 needed only 18 days (Fig. 5). Regarding the number of clusters, the most clusters plant^{-1}, with counts of 16.57 and 16.00 was observed in IVRP 11.5 and IVRP 11.6, respectively which are statically similar with parent (Fig. 5). Besides, IVRP 11.4 produced the fewest (7.00), whereas, the highest number of flowers cluster^{-1} (7.25) was recorded in IVRP 11.5. The result was significantly higher than parent Binatomato-11 (6.05) (Fig. 6).

Table 1. Comparison of leaf, flower and fruit traits in different tomato somaclonal variants

Genotype	Leafs' color	Leaflets' Orientation	Leaf Type	Leaf blades' shape	Presence of intercalary leaflet	Flowers' shape	Fruits' shape	Fruits' color
IVRP 11.3	Light green	Alternate	Typical tomato type	lance shaped with serrated edges	Absent	Star	Ovate	Red
IVRP 11.4	Light green	Alternate	Typical tomato type	lance shaped with serrated edges	Absent	Star	Ovate	Red
IVRP 11.5	Dark green	Alternate	Typical tomato type	lance shaped with serrated edges	Present	Star	Flattened	Red
IVRP 11.6	Light green	Alternate	Typical tomato type	lance shaped with serrated edges	Present	Star	Round	Red
Binatomato-11	Light Green	Alternate	Typical tomato type	lance shaped with serrated edges	Present	Star	Ellipsoid	Red
IVRP 13.1	Dark Green	Alternate	Typical tomato type	lance shaped with serrated edges	Present	Star	Flattened	Red
IVRP 13.2	Dark Green	Alternate	Potato type	Oval	Present	Star	Flattened	Red
IVRP 13.3	Dark Green	Opposite	Typical tomato type	lance shaped with serrated edges	Absent	Marigold	Flattened	Red
Binatomato-13	Light Green	Alternate	Typical tomato type	lance shaped with serrated edges	Present	Star	Round	Red

N.B: Data are collected according to Nakayama *et al*., 2023; Mata-Nicolás, *et al*., 2020; Roohanitaziani *et al*., 2020

When comparing the somaclones to the parent genotype, notable variations in yield characteristics were also observed. IVRP 11.6 yielded the highest yield plot^{-1} (32.16 kg) and second heaviest individual fruit (60.36 g), while IVRP 11.4 produced the lowest yield plot^{-1} (11.10 kg) and lightest fruit weight (33.03 g). On the other hand, the highest no. of fruit plant^{-1} was recorded (89.00) in IVRP 11.6, but IVRP 11.3 produced just 40.00. However, surprisingly, the individual fruit weight was the highest in IVRP 11.3 (67.44 g), which was significantly more than Binatomato-11. These findings is in agree with Anonymous (2020), which showed that the single fruit weight increase when the number of flowers cluster^{-1} was low (Fig. 6).

Additionally, the ratio of fruit length to breadth, ranging from 0.88 to 1.07 in case of *in vitro* regenerated plants derived from Binatomato-11, broadly represents the variation in fruit shape from ovate to round and cylindrical. In the parent, this ratio was 0.95 indicating ellipsoid (slightly elongated) shaped fruits. This result also affirms the visual characterization of fruits among the somaclonal genotypes (Fig. 1). Notably, genotype IVRP 11.4 exhibited a significant difference from the parent in this parameter, indicating an ovate shaped with sharp edges (Table 2). SUN, OVATE, LC, and FAS genes were associated with fruit shape, and mutations in these genes explain a large extent of the diversity in tomato fruit morphology (Rodríguez *et al*., 2011)

The total soluble solids content was recorded between 5.30 to 5.89 % brix among the genotypes. The highest TSS value was observed in IVRP 11.3 (5.89% brix), similar to that of the parent, presenting better fruit quality in terms sweetness and flavor. In contrast, the TSS content in IVRP 11.6 and IVRP 1.5 genotypes was lower than that of the parent. This variation suggests genetic differences in sugar metabolism and accumulation among these genotypes. However, rather than genetic factors alone, environmental conditions and interactions between genotype and environment can also influence the sugar accumulation (Zheng *et al*., 2023).

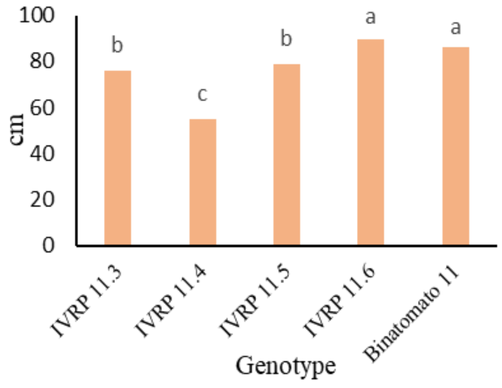

Fig. 3. Comparative analysis of plant height of somaclonal varients derived from Binatomato-11.

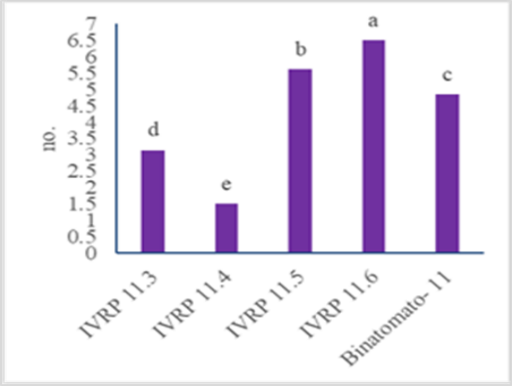

Fig. 4. Comparative analysis of no. of branches plant^{-1} of somaclonal varients derived from Binatomato-11.

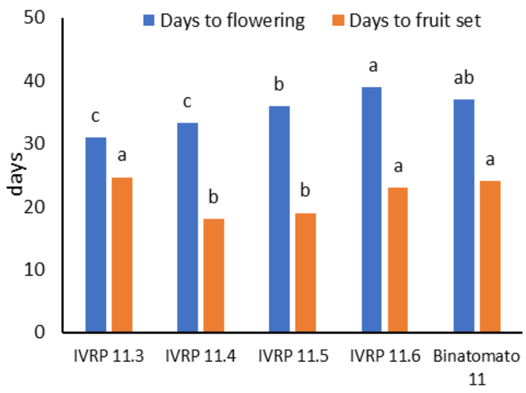

Fig. 5. Comparative analysis of days to 1st flowering and days to fruit set of somaclonal variants derived from Binatomato-11.

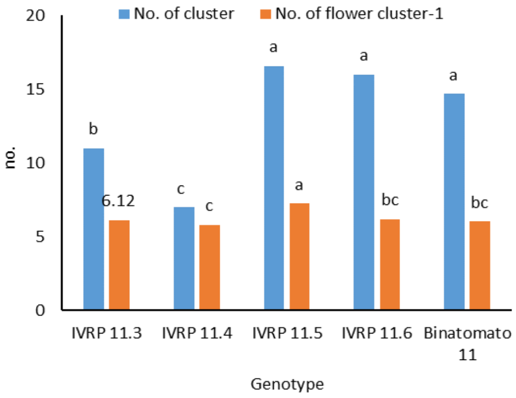

Fig. 6. Comparative analysis of no. of cluster plant^{-1} and no. of flowers cluster^{-1} of somaclonal variants derived from Binatomato-11.

Table 2. Comparative analysis on yield characteristics of somaclonal varients derived from Binatomato-11

Genotype	Weight of individual fruit (g)	No. of fruits plot^{-1}	Yield plot^{-1} (kg)	No. of fruits plant^{-1} (no.)	Length of fruit (cm)	Breadth of fruit (cm)	Length Breadth^{-1}	TSS (% brix)
IVRP 11.3	67.44 a	280 c	18.69 c	40.00 c	4.00 ab	4.25 a	0.94 b	5.89 a
IVRP 11.4	33.03 d	336 b	11.10 d	56.00 b	3.65 b	3.43 b	1.07 a	5.87 a
IVRP 11.5	47.47 c	502 a	23.82 b	83.67 a	4.21 a	4.59 a	0.92 b	5.65 b
IVRP 11.6	60.36 ab	534 a	32.16 a	89.00 a	3.99 ab	4.51 a	0.88 b	5.30 c
Binatomato-11	59.99 b	378 b	22.68 b	54.00 b	4.20 a	4.43 a	0.95 b	5.89 a
CV (%)	7.32	6.51	4.85	6.5	4.86	4.66	3.72	1.61
LSD 0.05	7.39	49.77	1.98	7.9	0.37	0.37	0.07	0.17

Among the somaclonal genotypes regenerated from Binatomato-13, the longest plant height was recorded in IVRP 13.2 (76.86 cm) but not more than parent genotype (80.11 cm) (Fig. 7). Conversely, IVRP 13.3 was the shortest genotype, measuring only 55.28 cm. The genotype IVRP 13.1 and IVRP 13.2 had the most branches, whereas the parent genotype had the fewest branches (Fig. 8). Compared to the other genotypes, IVRP 13.2 showed the significantly the shortest duration in terms of blooming and fruit set, needing fewer days to do so (Fig. 9). Moreover, the maximum no. of clusters plant^{-1}was recorded in IVRP 13.2, whereas IVRP 13.1 produced the highest number of flowers cluster^{-1}which is statistically significant from Binatomato-13 (Fig. 10). When analyzing the yield traits, surprisingly, the total number of fruit plot^{-1} was found the highest in IVRP 13.1 (711.67), but its individual fruit weight was the lowest (56.17 g) among the genotypes. Despite this, IVRP 13.1 recorded the highest yield plot^{-1} and no. of fruits plant^{-1} (39.86 kg and 107, respectively), significantly exceeding the performance of Binatomato-13, which had a yield plot^{-1} 11.00 kg and number of fruits plant^{-1} 21.00, respectively (Table 3). The analysis of fruit dimensions and total soluble sugar revealed that all of the *in vitro* regenerated genotypes were almost similar in total soluble sugar (Table 3) but significantly different from parent in fruit dimension. Fruit length ranged from 3.67 to 5.19 cm and breadth ranged from 4.80 to 5.66 cm. This result is close to the research findings of Yesmin *et al*. (2014), Patwary *et al*. (2013); Kouam *et al*. (2018) and Salim *et al*. (2018).

The occurrence of somaclonal variation can be attributed to both endogenous and exogenous factors, which are frequently closely linked to the mode of regeneration. The primary endogenous factor is the genotype of the mother plant used as the starting material for culture. *In vitro* genetic changes are influenced by exogenous influences, including temperature changes, light quality, artificial culture media with growth regulators, and different photoperiods. Additionally, plant growth regulators, the age of cultures, and the regeneration system are pivotal reasons for genetic alterations in controlled crop cultures. In particular, cells undergo a series of traumatic experiences throughout the disordered growth phase from tissue to callus, which could be a key contributing factor to somaclonal variation. Therefore, it is challenging to evaluate the role of each *in vitro* environmental factor, and occasionally such roles are unclear (Ferreira *et al*., 2023; Rai, 2021; Leva and Rinaldi, 2017). The genetic alterations among *in vitro* regenerated plants may be caused by single gene mutations (Leva and Rinaldi, 2017), transposable element activation (Bairu *et al*., 2011), and hypo- or hyper-methylation of DNA (Miguel and Marum, 2011; Evans and Sharp, 1983; Abdellatif *et al*., 2012). Kochieva *et al*. (2002), who observed such a mutability examining the genetic variability at the DNA level.

In contrast to genetic differences, epigenetic alterations, like DNA methylation, are often unstable can disappear within a few generations, or when plants are withdrawn from culture (Gulzar *et al*., 2020; Ali *et al*., 2018; Kaeppler *et al*., 2000; Bardini *et al*., 2003). Epigenetic variation is not desirable for plant breeding because it does not provide long term stability, although these variations can affect gene expression and reported to be more frequent (Leva and Rinaldi, 2017). On the other hand, genetic variation is heritable (Skirvin *et al*., 1994). In an experiment conducted by Plevneš *et al*., in 2007, it was found that *L. pennelli* callus exhibited resistance at the DNA level, but this resistance was not observed at the plant stage in the field under salt stress conditions. Therefore, the identification and confirmation of genetic changes are necessary for the successful application of somaclone in plant breeding. It has already been demonstrated that just slight genotypic changes can frequently result in significant phenotypic differences (Sacco *et al*., 2015).

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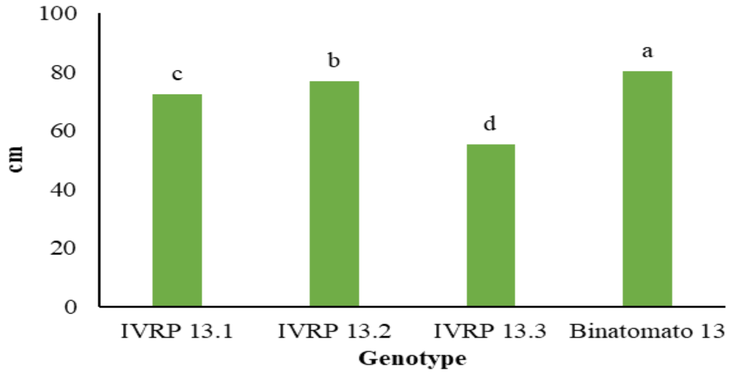

Fig. 7. Comparative analysis of plant height of somaclonal varients derived from Binatomato-13.

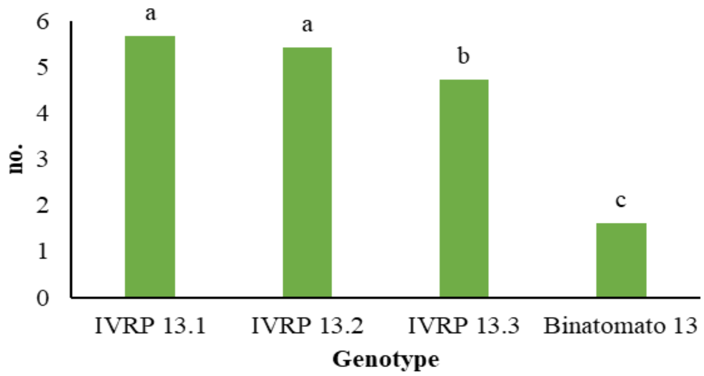

Fig. 8. Comparative analysis of no. of branches plant^{-1} of somaclonal varients derived from Binatomato-13.

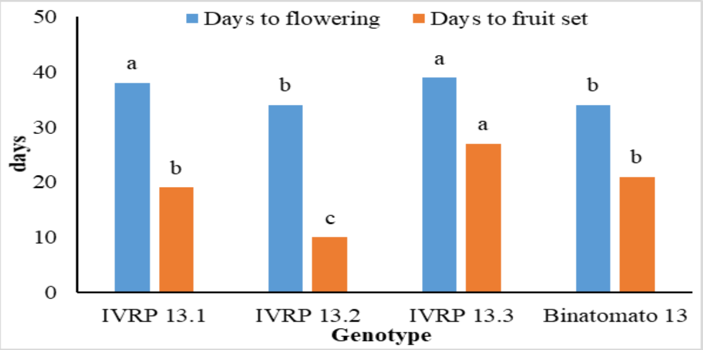

Fig. 9. Comparative analysis of days to 1st flowering and days to fruit set of somaclonal varients derived from Binatomato-13.

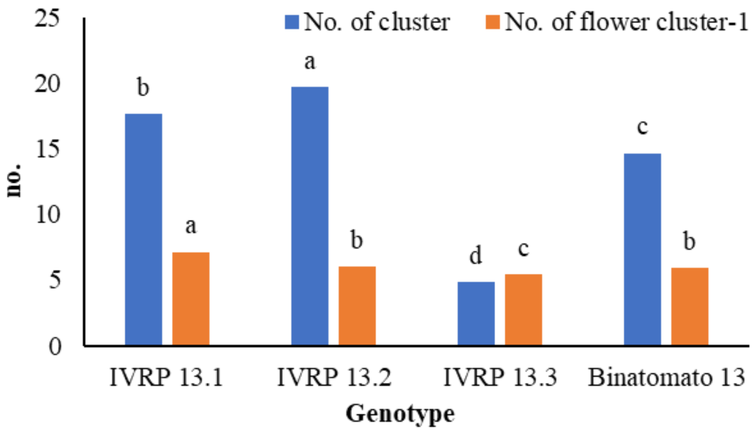

Fig. 10. Comparative analysis of no. of clusters plant^{-1} and no. of flowers cluster^{-1} derived from Binatomato-13.

Table 3. Comparative analysis on yield characteristics of somaclonal variants derived from Binatomato-13

Genotype	Weight of individual fruit (g)	No. of fruits plot^{-1}	Yield plot^{-1} (kg)	No. of fruits plant^{-1} (no.)	Length of fruit (cm)	Breadth of fruit (cm)	Length Breadth^{-1}	TSS (% brix)
IVRP 13.1	56.17 b	711.67 a	39.86 a	107 a	3.86 b	4.80 b	0.81 b	5.45
IVRP 13.2	61.65 b	348.00 b	21.47 b	58 b	4.06 b	5.66 a	0.72 c	5.29
IVRP 13.3	63.56 b	140.00 c	8.88 c	35 c	3.67 b	5.33 a	0.69 c	5.45
Binatomato-13	75.67 a	147.33 c	11.0 c	21 d	5.19 a	5.43 a	0.95 a	5.34
CV (%)	6.94	9.21	7.70	7.16	5.48	4.77	3.06	1.93
$LSD_{0.05}$	8.91	61.94	3.13	7.91	0.46	0.51	0.05	0.21

Conclusion

The investigated somaclones in this study possess characters that could be of interest to plant breeders. The characteristics of the flower and fruit of the tomato cultivars have been highlighted in this study as key descriptors of tomato. The observed diversity in morphological and yield-related traits provides a foundation for selecting genotypes with specific desirable attributes. *In vitro* regenerated somaclonal variants such as IVRP 11.6, IVRP 11.3, and IVRP 13.1 resulted in superior yield traits and fruit quality compared to their parent. In conclusion, the study disclosed that *in vitro* regeneration-induced somaclonal variation is a promising approach to generate diversity in tomatoes.

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