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Variability of agromorphological traits in *Portulaca grandiflora* through induced mutation using colchicine

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Abstract. *Aisyah SI, Meiningrum NI, Yudha YS, Nurcholis W. 2024. Variability of agromorphological traits in* Portulaca grandiflora *through induced mutation using colchicine. Biodiversitas 25: 2484-2493. Portulaca grandiflora* Hook is an ornamental plant originating from the Portulacaceae family, and it has been extensively cultivated on various continents. *P. grandiflora* displays appealing flower shapes and vibrant colors suitable for growth in a tropical landscape. Increased morphological diversity carries significant importance in the aesthetic function of ornamental plants. The higher the variation in the morphological characteristics of ornamental plants, the greater the market value of these plants due to their enhanced aesthetic appeal. Additionally, colchicine, a polyploidy-inducing agent, can enhance the plant genetic diversity. This study aims to investigate the impact of colchicine application on the morphological characters of *P. grandiflora*. The experiment was conducted for four months at the Leuwikopo Experimental Field, IPB, with two factors of Completely Randomized Block Design: The colchicine treatment comprises five concentration levels (0, 0.05, 0.1, 0.15, and 0.2), with four test genotypes: Orange, Peach, Bicolor, and Banana. A significant reduction in growth was observed in the Bicolor genotype, followed by the Banana genotype, indicating varying tissue sensitivities to colchicine treatment. The study reveals variations in morphological responses for each concentration treatment and genotype used. Statistical results confirm aspects: (i) each genotype displays different performance, (ii) the combined treatment of colchicine significantly influences the growth and development of quantitative traits, and (iii) The interaction (colchicine x genotype) was statistically significant for all quantitative characters of *P. grandiflora* except stem diameter. Qualitative traits, such as morphological changes in stem color, leaf color, and shape, were observed. Three correlation values are positive and statistically significant: plant length and leaf width, number of branches on shoots and leaves, and number of branches on shoots and flowers. Putative mutants of *P. grandiflora* exhibiting new traits require in-depth exploration in subsequent generations.

Keywords: Morphological variations, ornamental crop, polyploidy induction

INTRODUCTION

Portulaca grandiflora Hook. belongs to the ornamental plant species within the Portulacaceae family. In addition to its role as an ornamental plant, *P. grandiflora* is also recognized for its medicinal properties due to its biochemical constituents. All parts of the *P. grandiflora* plant can be consumed for medicinal treatments. In addition to flavonoids, polyphenols, sterols, and polysaccharides, which are distributed throughout the whole plant, betalains are essential compounds found in the flower of *P. grandiflora* (Zhou et al. 2015). These unique water-soluble pigments are responsible for the vibrant colors in various fruits, vegetables, and flowers. Moreover, they offer health benefits to humans (Spórna-Kucab et al. 2022). Approximately 70 out of 100 species from the *Portulacaceae* family have been identified and studied intensively, and one of them is *P. grandiflora*. *P. grandiflora* is frequently applied in ornamental landscapes due to its creeping growth habit and rapid multiplication capacity (Husnawati et al. 2020); it is a succulent dicotyledon. Common morphological characteristics of *P. grandiflora* are reddish-green leaf color, with short round leaves ranging from 2-4 cm, flower diameter between 3-5 cm, and plant length ranging from 7-15 cm (Cruz et al. 2019). *Portulaca grandiflora* flowers are bisexual, with both actinomorphic and zygomorphic arrangements. They have bright-colored petals, numbering 4-5, while the sepals are typically around 2 (Setiawan et al. 2016). Although initially considered a weed due to its rapid and sprawling growth, the captivating beauty of the flower color of *P. grandiflora* and its shapes has led to its cultivation as an ornamental plant.

Ornamental plants emphasize beauty, so the main potential of ornamental plants lies in their unique and attractive morphology. Therefore, to enhance the appearance of ornamental plants with new variations of high aesthetic value, efforts are required to increase the plant genetic diversities. Genetic variations are crucial in plant breeding programs to produce novel superior cultivars (Patel et al. 2023). Artificial mutation is one of the methods used in plant breeding programs to enhance plant genetic diversity. The mutation alters genetic material at the genome, chromosome, or DNA level, resulting in novel genetic diversity (Chaudhary et al. 2019). Chemical mutagenic agents, including colchicine, can induce artificial mutation inductions. Colchicine ($C_{22}H_{25}NO_6$) is the most commonly used chemical for plant polyploidy manipulation. Colchicine is a toxic alkaloid compound with carcinogenic properties obtained from the extract of *Colchicum autumnale* (Eng and Ho 2019). Its action primarily inhibits the formation of spindle fibers during the anaphase process and impedes cell division during anaphase (both in mitosis and meiosis). By inhibiting spindle formation during division, chromosomes that have already duplicated are not separated in opposite directions, forming polyploid cells (Samatadze et al. 2022; Narukulla et al. 2023).

Various studies have indicated morphological changes resulting from colchicine application. Arindyaswari et al. (2022) reported that different levels of colchicine treatment led to some chromosome changes and alterations in plant morphology, as indicated by an increase in leaf size, stem diameter, and plant height on *Amaranthus tricolor* L. Colchicine induction in in-vitro cultured *Neolamarckia cadamba* resulted in the transformation of leaf shape and (Eng et al. 2021). Fathurrahman et al. (2023) reported a significant increase in plant height, pod weight, number of pods, and pod length in *Vigna unguiculata* var. *sesquipedalis* mutants caused by artificial colchicine-induced mutation. Besides increasing the diversity of quantitative traits, colchicine induces plant color and morphological shape changes. Samatadze et al. (2022) reported that tetraploid *Polemonium caeruleum*, induced through colchicine polyploidization, exhibited variations in leaf and flower shapes and colors. The diversity resulting from artificial mutation induced by colchicine has attracted the interest of ornamental plant breeders to obtain extensive genetic and morphological variations (Cabahug et al. 2020). Based on the literature review, research on mutation induction using colchicine in *P. grandiflora* is still limited. Hence, this study aims to evaluate the performance of agromorphological traits in several genotypes of *P. grandiflora* under various levels of colchicine treatment concentrations.

MATERIALS AND METHODS

Study site

This research was conducted at Leuwikopo Experimental field, Department of Agronomy and Horticulture, IPB University, Bogor, Indonesia.

Experimental design

The research used a Factorial Completely Randomized Design (FCRD) with two factors. The first factor involved colchicine at five concentrations (0%, 0.05%, 0.1%, 0.15%, and 0.2%). The second factor comprised four distinct *P. grandiflora* genotypes: Orange, Peach, Bicolor, and Banana (Figure 1). This design yielded 20 treatment combinations, each replicated three times, resulting in 60 experimental units. Each replication consisted of 3 plants, totaling 180 observed plants. The statistical model utilized is as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} : Observed value for the i-th level of colchicine concentration, j-th level of genotype, and k-th replication.

μ : Overall mean of the observations.

α_i : Main effect of colchicine concentration at level i (1, 2, 3, 4, 5).

β_j : Main effect of genotype at level j (1, 2, 3, 4).

$(\alpha\beta)_{ij}$: Interaction effect between colchicine concentration level i and genotype level j.

ε_{ijk} is the random experimental error, which is assumed to be normally distributed, associated with the i-th level of colchicine concentration, the j-th level of genotype, and the k-th replication.

Planting

Planting was carried out using vegetative cuttings from prepared seedlings. These stem cuttings were planted in 18 cm x 18 cm polybags filled with growing medium. The growth medium comprises soil, manure, and potting soil in a 1:1:1 ratio. Shade was provided for one week before treatment to allow the planted *P. grandiflora* to adapt to the ambient temperature and initiate root development.

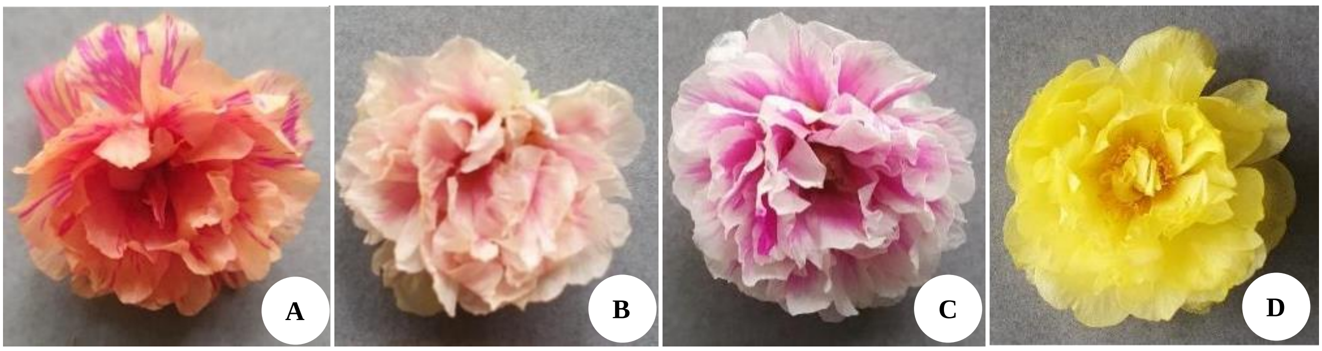

Figure 1. Color and shape of *Portulaca grandiflora* Hook flowers: A. Orange genotype; B. Peach genotype; C. Bicolor genotype; D. Banana genotype

Colchicine application

Colchicine was applied to one-week-old *P. grandiflora* plants with already-developed roots. The growing tips were pruned to promote the growth of lateral shoots. Every bud was treated with two drops of colchicine in the morning. The treated shoots were covered with transparent plastic for three days to prevent colchicine evaporation. The treated shoots were the third ones from the pruning point; each received its respective label. Untreated shoots were pruned to reduce nutrient competition. The treated plants remained shaded for four days before being transferred to a well-lit area. *Portulaca grandiflora* plants were cultivated in polybags on silver mulch.

Plant maintenance

The *P. grandiflora* plants were watered twice daily when the soil became dry and once when the growing medium was moist. Watering was done manually using a watering can. Weed management involved periodic weeding, typically performed once a week based on weed growth intensity. Weeding is done regularly, usually once a week, depending on weed growth to ensure the health of the *P. grandiflora* plant. Routine maintenance continued for 12 weeks after colchicine treatment.

Observation of plant morphology

Plant morphology was observed on all 60 experimental units. Each plant with colchicine treatment was compared to the control. Quantitative characteristics of plant morphology that were observed included: (i) Plant height (cm): Plant height was measured from the stem's base to the point where flowers emerged. (ii) Plant length (cm): Measured from the base to the tip of the third shoot. (iii) Leaf length (cm): measured from the leaf's base to the tip of the third shoot. (iv) Leaf width (cm): Measured on the widest point of the leaf. (v) Stem diameter (cm): Measured at one-third of the plant's height using calipers. (vi) Number of leaves: by counting all the leaves on the third shoot. (vii) Number of flowers: by counting the number of flowers. (viii) Number of branches on shoots: counting all branches on the third shoot.

Qualitative characteristics include: (i) Stem color was observed during the maximum vegetative phase. (ii) Leaf color was observed on the entire leaf in the third shoot. (iii) Flower color was observed on the flowers on the third shoot. (iv) Leaf shape was observed on leaves on the third shoot. (v) Flower shape was observed on the flowers on the third shoot.

Data analysis and visualization

Analysis of Variance (ANOVA) was conducted utilizing Microsoft Excel and the Statistical Analysis System (SAS) software at a 5% significance level and the Duncan Multiple Range Test (DMRT) for post hoc comparisons with a confidence interval of α=0.05 for quantitative variables. Qualitative data were evaluated descriptively by comparing the characteristics of the control plants. Leaf, stem, and flower colors were observed using a mini RHCC. Growth rate charts were created using R studio software with the 'ggplot2' package. A correlation plot was created using R studio packages 'corrplot' and 'psych.'

RESULTS AND DISCUSSION

Growth rate

Figure 2 displays the growth percentage of the tested genotypes of *P. grandiflora* through colchicine treatment. The four genotypes had different growth responses. The growth capabilities of the four tested genotypes of *P. grandiflora* differed at each colchicine treatment level. In the orange genotype, the lowest growth percentage (77.78%) was found at concentrations of 0.05%, 0.10%, and 0.20%. Meanwhile, the lowest growth percentage of the peach genotype was at 0.15% and 0.20% (66.67%). There are differences in the growth capabilities of the five concentrations of colchicine. The growth of the Bicolor genotype decreased as the colchicine concentration increased, and the lowest growth percentage (44.44%) was obtained at the highest colchicine treatment concentration (0.20%). On the other hand, the lowest growth rate percentage in Banana genotypes was obtained at a concentration of 0.15% (44.44%).

Plant height

The effect of colchicine treatment on plant height was observed from the 2nd to the 12th week (Table 1). The colchicine treatment resulted in differences in plant height growth variations. Regarding genetic potential, the tested genotypes of *P. grandiflora* exhibit diverse growth performance. The K4G3 treatment had the highest average plant height (18.53) in the 10th week, followed by the K1G3 treatment (14.60). The impact of the colchicine treatment on plant height varies. Some treatments lead to an increase in plant height, while others result in a decrease. All treatments exhibited varying trends in plant height over the period, except for the K4G4 treatment, which displayed a consistent reduction until the final week of observation.

Plant length

The length of *P. grandiflora* plants was measured over six weeks from week 6th to week 12th (Table 2). Genetically, the Orange genotype (K0G1) exhibited the most extended plant length at week 12, while the K3G4 treatment had the lowest. The results showed that colchicine treatment caused morphological changes in *P. grandiflora* at week 12. From the sixth to the tenth week, all treatments had no significant changes in plant length. The shortest plants (10.57 cm) were in the K3G4 treatment.

Table 1. Effect of colchicine treatment on plant height

Treatments	Week after treatment					
	2	4	6	8	10	12
KOG1	6.61 ^{d}	7.24 $^{a-d}$	7.33 $^{b-e}$	11.49 ^{ab}	13.45^{ab}	13.44^{ab}
KOG2	9.62 $^{a-d}$	7.46 $^{a-d}$	5.86 $^{c-e}$	6.50 ^{ab}	11.53^{ab}	13.43 ^{ab}
KOG3	10.71 $^{a-c}$	10.18 ^{a}	10.30 ^{ab}	10.80 ^{ab}	10.26^{ab}	13.29 ^{ab}
KOG4	6.95 ^{d}	5.45 ^{d}	5.67 $^{c-e}$	5.97 ^{ab}	6.12^{ab}	12.47 ^{ab}
K1G1	7.09 ^{d}	6.40 $^{b-d}$	5.62 $^{c-e}$	6.00 ^{ab}	6.37^{ab}	12.39 ^{ab}
K1G2	11.03 ^{ab}	7.85 $^{a-d}$	7.02 $^{b-e}$	6.60 ^{ab}	6.81^{ab}	12.65 ^{ab}
K1G3	8.56 $^{a-d}$	9.31 $^{a-c}$	8.43 $^{b-d}$	12.13 ^{ab}	14.60^{ab}	13.76^{a}
K1G4	8.78 $^{a-d}$	7.33 $^{a-d}$	5.89 $^{c-e}$	5.73 ^{b}	8.33^{ab}	12.70 ^{ab}
K2G1	7.30 ^{d}	6.01 ^{cd}	6.79 $^{b-e}$	8.14 ^{ab}	8.41^{ab}	12.72 ^{ab}
K2G2	7.67 ^{cd}	4.62 ^{d}	4.88 ^{de}	5.72 ^{b}	4.84^{b}	12.40 ^{ab}
K2G3	9.67 $^{a-d}$	10.32 ^{a}	9.11 $^{a-c}$	9.81 ^{ab}	9.52^{ab}	13.19 ^{ab}
K2G4	8.39 $^{a-d}$	5.22 ^{d}	5.39 $^{c-e}$	9.62 ^{ab}	11.12^{ab}	12.12 ^{ab}
K3G1	8.15 $^{b-d}$	4.48 ^{d}	5.78 $^{c-e}$	5.74 ^{b}	5.51^{b}	12.51 ^{ab}
K3G2	7.87 ^{cd}	5.35 ^{d}	5.18 ^{de}	5.12 ^{b}	5.31^{b}	11.89^{b}
K3G3	11.37 ^{a}	9.96 ^{a}	11.96 ^{a}	13.33 ^{a}	12.97^{ab}	13.93^{a}
K3G4	8.80 $^{a-d}$	7.26 $^{a-d}$	4.45 ^{e}	4.82 ^{b}	4.76^{b}	12.46 ^{ab}
K4G1	8.16 $^{b-d}$	4.36 ^{d}	5.12 ^{de}	6.92 ^{ab}	10.31^{ab}	13.09 ^{ab}
K4G2	7.23 ^{d}	4.97 ^{d}	4.78 ^{de}	5.00 ^{b}	5.02^{b}	11.87^{b}
K4G3	9.36 $^{a-d}$	9.66 ^{ab}	7.47 $^{b-e}$	12.42 ^{ab}	18.53^{a}	12.47^{ab}
K4G4	8.35 $^{b-d}$	5.97 ^{cd}	5.50 $^{c-e}$	5.13 ^{b}	5.13^{b}	2.63^{ab}

Note: Numbers followed by different letters in the same column indicate significant differences based on the 5% DMRT test. The data represent the mean value of plant height for each week. The symbol 'K' denotes concentration: K1 (0.05%), K2 (0.1%), K3 (0.15%), and K4 (0.2%). 'G' represents genotypes: G1 (Orange), G2 (Peach), G3 (Bicolor), and G4 (Banana). Plant height was measured in centimeters (cm)

Table 2. Effect of colchicine treatment on plant length

Treatments	Week after treatment			
	6	8	10	12
KOG1	11.37^{a}	13.79^{a}	17.19^{a}	22.93^{a}
KOG2	11.23^{a}	12.24^{a}	13.80^{a}	13.60 $^{a-c}$
KOG3	10.65^{a}	11.78^{a}	14.11^{a}	12.25 $^{a-c}$
KOG4	12.33^{a}	15.92^{a}	17.58^{a}	19.40 $^{a-c}$
K1G1	12.31^{a}	13.76^{a}	14.50^{a}	16.27 $^{a-c}$
K1G2	12.78^{a}	17.22^{a}	18.72^{a}	18.20 $^{a-c}$
K1G3	12.07^{a}	15.86^{a}	17.66^{a}	18.76 $^{a-c}$
K1G4	8.58^{a}	12.56^{a}	12.14^{a}	14.01 $^{a-c}$
K2G1	8.83^{a}	12.89^{a}	14.66^{a}	19.22 $^{a-c}$
K2G2	12.06^{a}	12.17^{a}	16.26^{a}	18.67 $^{a-c}$
K2G3	12.42^{a}	16.89^{a}	18.67^{a}	17.62 $^{a-c}$
K2G4	12.78^{a}	15.16^{a}	17.02^{a}	18.06 $^{a-c}$
K3G1	12.73^{a}	14.44^{a}	16.82^{a}	18.88 $^{a-c}$
K3G2	13.87^{a}	15.21^{a}	15.56^{a}	17.18 $^{a-c}$
K3G3	8.65^{a}	12.00^{a}	12.86^{a}	13.07^{bc}
K3G4	7.75^{a}	10.58^{a}	10.13^{a}	10.57^{c}
K4G1	7.65^{a}	12.36^{a}	13.33^{a}	16.67 $^{a-c}$
K4G2	15.08^{a}	17.39^{a}	18.71^{a}	21.41^{ab}
K4G3	14.25^{a}	15.83^{a}	16.17^{a}	21.33^{ab}
K4G4	11.38^{a}	12.13^{a}	13.00^{a}	16.65 $^{a-c}$

Note: Numbers followed by different letters in the same column indicate statistically significant differences based on the 5% DMRT test. The data represent the mean value of plant length for each week. The symbol 'K' denotes concentration: K1 (0.05%), K2 (0.1%), K3 (0.15%), and K4 (0.2%). 'G' represents genotypes: G1 (Orange), G2 (Peach), G3 (Bicolor), and G4 (Banana). Plant length was measured in centimeters (cm)

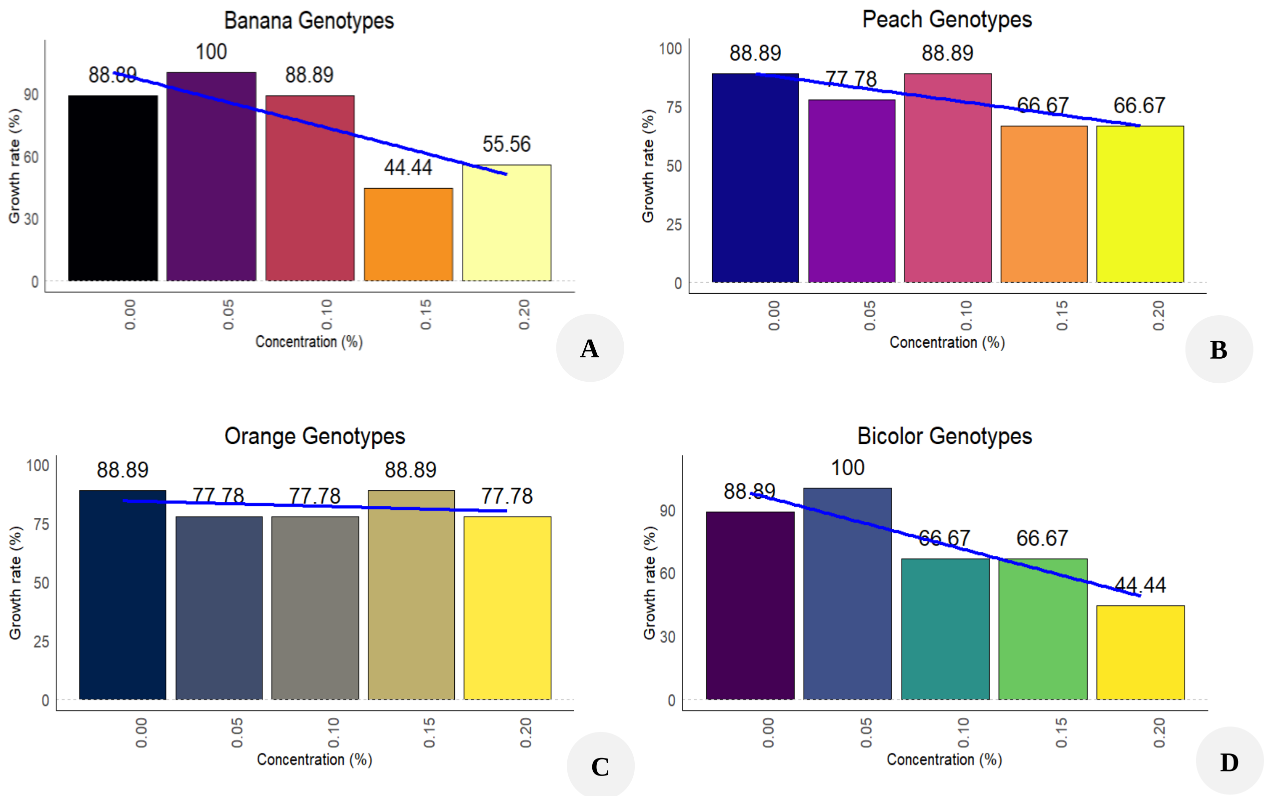

Figure 2. The growth percentages of four *Portulaca grandiflora* genotypes: A. Banana genotype; B. Peach genotype; C. Orange genotype, and D. Bicolor genotype

Leaf length

Colchicine treatments affected *P. grandiflora* leaf length (Table 3). The treatments of K0G1-K0G4, not treated with colchicine, had similar leaf lengths. In weeks 6-12, K3G4 exhibited the broadest leaves compared to these untreated genotypes. Conversely, during the sixth, eighth, and twelfth weeks, treatments K3G3, K1G4, and K4G1 had the shortest leaf lengths.

Leaf width

Table 4 presents the leaf width for colchicine-genotype combination treatments from week 6 to week 12. Notably, the orange genotype (K0G1) had the widest leaves by the 12th week, while the K0G4 treatment had the narrowest. Significant differences in leaf width were observed in the sixth week, with K0G2 having the broadest and K3G4 (0.22) being the narrowest. However, the eighth week of treatment showed no significant effect of colchicine on leaf width. In the week of the 10th and 12th, K0G1 had the widest leaves, indicating that colchicine significantly affected leaf width. In contrast, the K3G4 treatment (Banana genotype with 0.15% colchicine) had the narrowest leaves.

Stem diameter

At week 8th, stem diameters across all treatments ranged from 0.6 to 1.1 cm, increasing from 1.0 to 1.6 cm by the week 12th. The Banana genotype treated with 0.2% colchicine (K4G4) showed increased stem diameter from 1.12 cm at week 8 to 3.05 cm at week 12th.

Number of leaves

Table 5 shows the effect of the treatment combination of colchicine concentrations and genotype on the average number of leaves in each treatment. The orange genotype (K0G1) had the highest number of leaves, followed by the banana genotype (K0G3) and the bicolor genotype (K0G4). The treatments induced substantial variations in the number of leaves. In week six, the mean value for number of leaves was between 22.78 (K1G4) and 64.83 (K2G4). In week eight, the number of leaves varied from 28.56 (K3G3) to 118.39 (K4G2). In week ten, the K3G2 treatment had a significantly different average number of leaves of 149.39 compared to the K3G3 treatment (30.56) and the K4G4 treatment (36.25). In week twelve, the K1G1 treatment had the highest number of leaves, while the K3G3 treatment had the fewest.

Number of flowers

Table 6 presents the number of flowers in *P. grandiflora* due to the combination of colchicine and genotype treatments. Control treatment on 4 genotypes showed that the number of flowers in the KOG3 treatment increased in week 10 (2.00) but decreased by week 12. The number of flowers in the KOG4 treatment was 3.83 in week 8 but decreased by week 12. In weeks 6 and 8, all treatments were not significantly different, indicating that the combined colchicine-genotype treatments had no significant impact on the number of flowers. In week 10, the K1G2 treatment had the highest number of flowers, significantly different from the treatments of KOG1-KOG4). In week 12, the K3G2 treatment had the highest number of flowers and significantly differed from the treatments of KOG2-KOG4.

Table 3. Effect of colchicine treatment on leaf length

Treatments	Week after treatment			
	6	8	10	12
KOG1	2.11^{b}	2.28^{ab}	2.42^{b}	2.60^{b}
KOG2	2.11^{b}	2.17^{ab}	2.36^{b}	2.41^{b}
KOG3	2.02^{b}	2.07^{b}	2.01^{b}	2.17^{b}
KOG4	2.31^{b}	2.39^{ab}	2.41^{b}	2.43^{b}
K1G1	2.17^{b}	2.22^{ab}	2.34^{b}	2.58^{b}
K1G2	2.22^{b}	2.37^{ab}	2.39^{b}	2.43^{b}
K1G3	2.37^{b}	4.53^{ab}	2.47^{b}	2.51^{b}
K1G4	1.96^{b}	2.16^{ab}	2.28^{b}	2.29^{b}
K2G1	2.02^{b}	2.27^{ab}	2.21^{b}	2.37^{b}
K2G2	2.16^{b}	2.40^{ab}	2.13^{b}	2.45^{b}
K2G3	2.24^{b}	2.37^{ab}	2.55^{b}	2.58^{b}
K2G4	2.34^{b}	2.20^{ab}	2.00^{b}	2.31^{b}
K3G1	2.27^{b}	2.31^{ab}	2.29^{b}	2.37^{b}
K3G2	2.44^{b}	2.38^{ab}	2.53^{b}	2.60^{b}
K3G3	1.87^{b}	1.97^{b}	2.14^{b}	2.03^{b}
K3G4	3.22^{a}	4.78^{a}	5.62^{a}	7.49^{a}
K4G1	1.89^{b}	2.14^{ab}	2.16^{b}	2.17^{b}
K4G2	2.23^{b}	2.77^{ab}	2.41^{b}	2.83^{b}
K4G3	2.20^{b}	2.28^{ab}	2.40^{b}	2.50^{b}
K4G4	2.03^{b}	2.10^{b}	2.13^{b}	2.62^{b}

Note: Numbers followed by different letters in the same column indicate statistically significant differences based on the 5% DMRT test. The data represent the mean value of leaf length for each week. The symbol 'K' denotes concentration: K1 (0.05%), K2 (0.1%), K3 (0.15%), and K4 (0.2%). 'G' represents genotypes: G1 (Orange), G2 (Peach), G3 (Bicolor), and G4 (Banana). Leaf length was measured in centimeters (cm)

Table 4. Effect of colchicine treatment on leaf width

Treatments	Week after treatment			
	6	8	10	12
KOG1	0.26^{b}	0.29^{a}	0.32^{a}	0.35^{a}
KOG2	0.72^{a}	0.26^{ab}	0.26^{a-c}	0.29^{ab}
KOG3	0.23^{b}	0.22^{ab}	0.22^{bc}	0.27^{bc}
KOG4	0.27^{b}	0.28^{a}	0.38^{a-c}	0.28^{b}
K1G1	0.22^{b}	0.27^{ab}	0.30^{a}	0.30^{ab}
K1G2	0.27^{b}	0.27^{ab}	0.31^{a}	0.32^{ab}
K1G3	0.23^{b}	0.26^{ab}	0.27^{a-c}	0.29^{ab}
K1G4	0.22^{b}	0.27^{ab}	0.27^{a-c}	0.28^{b}
K2G1	0.24^{b}	0.27^{ab}	0.28^{a-c}	0.29^{ab}
K2G2	0.26^{b}	0.28^{a}	0.29^{ab}	0.30^{ab}
K2G3	0.23^{b}	0.30^{a}	0.31^{a}	0.31^{ab}
K2G4	0.32^{b}	0.28^{ab}	0.29^{ab}	0.31^{ab}
K3G1	0.26^{b}	0.28^{ab}	0.29^{ab}	0.29^{ab}
K3G2	0.28^{b}	0.27^{ab}	0.30^{a}	0.30^{ab}
K3G3	0.19^{b}	0.23^{ab}	0.26^{a-c}	0.27^{bc}
K3G4	0.20^{b}	0.23^{ab}	0.22^{c}	0.22^{c}
K4G1	0.33^{b}	0.25^{ab}	0.30^{a}	0.30^{ab}
K4G2	0.30^{b}	0.30^{a}	0.30^{a}	0.30^{ab}
K4G3	0.28^{b}	0.28^{a}	0.30^{a}	0.32^{ab}
K4G4	0.24^{b}	0.20^{b}	0.30^{a}	0.30^{ab}

Note: Numbers followed by different letters in the same column indicate statistically significant differences based on the 5% DMRT test. The data represent the mean value of leaf width for each week. The symbol 'K' denotes concentration: K1 (0.05%), K2 (0.1%), K3 (0.15%), and K4 (0.2%). 'G' represents genotypes: G1 (Orange), G2 (Peach), G3 (Bicolor), and G4 (Banana). Leaf width was measured in centimeters (cm)

Table 5. Effect of colchicine treatment on the number of leaves

Treatments	Week after treatment			
	6	8	10	12
KOG1	55.78^{ab}	78.22^{ab}	117.17^{ab}	13.57^{ab}
KOG2	47.95^{ab}	51.72^{ab}	51.33^{ab}	8.10^{b}
KOG3	33.83^{ab}	37.67^{b}	69.11^{ab}	10.39^{ab}
KOG4	25.45^{b}	32.39^{b}	62.61^{ab}	11.47^{ab}
K1G1	44.83^{ab}	79.50^{ab}	120.78^{ab}	15.13^{a}
K1G2	51.50^{ab}	56.28^{ab}	75.61^{ab}	8.59^{ab}
K1G3	39.06^{ab}	61.89^{ab}	73.33^{ab}	9.36^{ab}
K1G4	22.78^{b}	44.11^{ab}	44.11^{ab}	10.10^{ab}
K2G1	53.67^{ab}	79.00^{ab}	110.00^{ab}	10.23^{ab}
K2G2	37.72^{ab}	87.39^{ab}	113.33^{ab}	10.65^{ab}
K2G3	40.11^{ab}	80.28^{ab}	95.89^{ab}	10.47^{ab}
K2G4	64.83^{a}	82.55^{ab}	95.11^{ab}	11.40^{ab}
K3G1	35.28^{ab}	57.83^{ab}	72.39^{ab}	11.40^{ab}
K3G2	45.17^{ab}	58.44^{ab}	149.39^{a}	13.84^{ab}
K3G3	26.67^{ab}	28.56^{b}	30.56^{b}	7.99^{b}
K3G4	25.75^{b}	86.00^{ab}	103.83^{ab}	9.55^{ab}
K4G1	45.42^{ab}	53.33^{ab}	72.50^{ab}	11.86^{ab}
K4G2	41.33^{ab}	118.39^{a}	93.44^{ab}	12.74^{ab}
K4G3	45.00^{ab}	52.17^{ab}	72.78^{ab}	10.32^{ab}
K4G4	26.50^{ab}	33.00^{b}	36.25^{b}	11.77^{ab}

Note: Numbers followed by different letters in the same column indicate statistically significant differences based on the 5% DMRT test. The data represent the mean value of the number of leaves each week. The symbol 'K' denotes concentration: K1 (0.05%), K2 (0.1%), K3 (0.15%), and K4 (0.2%). 'G' represents genotypes: G1 (Orange), G2 (Peach), G3 (Bicolor), and G4 (Banana)

Table 6. Effect of colchicine treatment on the number of flowers

Treatments	Week after treatment			
	6	8	10	12
KOG1	1.00^{a}	1.33^{a}	1.50^{c}	1.54^{ab}
KOG2	1.00^{a}	1.44^{a}	1.50^{c}	1.02^{b}
KOG3	1.00^{a}	1.00^{a}	2.00^{bc}	1.03^{b}
KOG4	1.00^{a}	3.83^{a}	1.83^{c}	0.88^{b}
K1G1	1.00^{a}	3.50^{a}	3.67^{a-c}	1.54^{ab}
K1G2	1.00^{a}	1.50^{a}	5.00^{a}	1.38^{ab}
K1G3	1.00^{a}	1.22^{a}	2.22^{a-c}	1.11^{b}
K1G4	1.00^{a}	1.78^{a}	2.11^{bc}	1.17^{b}
K2G1	1.00^{a}	1.17^{a}	4.72^{ab}	1.52^{ab}
K2G2	1.00^{a}	1.50^{a}	3.89^{a-c}	1.42^{ab}
K2G3	1.00^{a}	1.33^{a}	1.89^{bc}	1.18^{b}
K2G4	1.00^{a}	2.28^{a}	3.61^{a-c}	0.91^{b}
K3G1	1.00^{a}	1.78^{a}	2.78^{a-c}	1.12^{b}
K3G2	1.00^{a}	1.67^{a}	2.70^{a-c}	2.69^{a}
K3G3	1.00^{a}	2.17^{a}	1.33^{c}	0.85^{b}
K3G4	1.00^{a}	1.00^{a}	2.00^{bc}	1.66^{ab}
K4G1	1.00^{a}	3.50^{a}	2.50^{a-c}	0.94^{b}
K4G2	1.00^{a}	2.83^{a}	2.96^{a-c}	1.56^{ab}
K4G3	1.00^{a}	2.33^{a}	1.50^{c}	0.97^{b}
K4G4	1.00^{a}	2.00^{a}	1.75^{c}	1.39^{ab}

Note: Numbers followed by different letters in the same column indicate statistically significant differences based on the 5% DMRT test. The data represent the mean value of the number of flowers each week. The symbol 'K' denotes concentration: K1 (0.05%), K2 (0.1%), K3 (0.15%), and K4 (0.2%). 'G' represents genotypes: G1 (Orange), G2 (Peach), G3 (Bicolor), and G4 (Banana)

Table 7. Effect of colchicine treatment on the number of branches on shoots

Treatments	Week after treatment			
	6	8	10	12
KOG1	3.61^{a-c}	5.33^{ab}	5.67^{a}	3.06^{a}
KOG2	3.28^{a-c}	4.92^{ab}	4.00^{a}	2.59^{ab}
KOG3	3.00^{a-c}	2.67^{b}	4.44^{a}	1.86^{ab}
KOG4	2.00^{bc}	2.78^{b}	4.72^{a}	2.20^{ab}
K1G1	4.39^{a-c}	7.61^{ab}	5.00^{a}	3.14^{a}
K1G2	3.39^{a-c}	3.39^{ab}	6.06^{a}	2.06^{ab}
K1G3	3.17^{a-c}	5.25^{ab}	8.78^{a}	2.42^{ab}
K1G4	6.50^{a-c}	6.67^{ab}	4.50^{a}	2.04^{ab}
K2G1	4.67^{a-c}	6.50^{ab}	11.00^{a}	3.02^{a}
K2G2	8.33^{ab}	8.83^{a}	9.50^{a}	1.98^{ab}
K2G3	1.17^{c}	4.22^{ab}	3.11^{a}	2.11^{ab}
K2G4	4.50^{a-c}	5.33^{ab}	5.33^{a}	2.16^{ab}
K3G1	3.00^{a-c}	3.39^{ab}	5.56^{a}	2.25^{ab}
K3G2	2.50^{a-c}	5.25^{ab}	7.67^{a}	2.18^{ab}
K3G3	9.50^{a}	4.25^{ab}	2.00^{a}	1.28^{b}
K3G4	5.00^{a-c}	6.67^{ab}	9.00^{a}	1.27^{b}
K4G1	2.33^{bc}	4.67^{ab}	5.83^{a}	1.71^{ab}
K4G2	5.83^{a-c}	4.67^{ab}	5.72^{a}	2.13^{ab}
K4G3	3.00^{a-c}	3.17^{b}	4.00^{a}	1.45^{b}
K4G4	1.00^{c}	6.00^{ab}	5.00^{a}	1.77^{ab}

Note: Numbers followed by different letters in the same column indicate statistically significant differences based on the 5% DMRT test. The data represent the mean value of weekly branches on shoots. The symbol 'K' denotes concentration: K1 (0.05%), K2 (0.1%), K3 (0.15%), and K4 (0.2%). 'G' represents genotypes: G1 (Orange), G2 (Peach), G3 (Bicolor), and G4 (Banana)

Number of branches on shoots

Table 7 presents the means for the number of branches on shoots from week 6 to week 12. The mean number of branches on shoots in week 6 ranged from 1.00 (K4G4) to 9.50 (K3G3). The K3G3 treatment significantly differs from the KOG4 treatment. Treatment K2G2 had the highest number of branches on shoots in week 8, while K4G3 treatment had the lowest. In week 12, treatment K1G1 exhibited the highest number of branches, whereas treatment K3G4 had the lowest.

Stem color

Figure 3 captures the change in stem color due to the application of colchicine. The results showed the changes in stem color in the Orange genotype after being treated with 0.1% and 0.2% colchicine. For the Peach genotype, the changes in stem color occurred at the treatment of 0.05% colchicine. The changes in stem color in the Bicolor and Banana genotypes occurred at the treatment of 0.15%.

Leaf color and shape

The application of colchicine triggered the changes in leaf shape but not in leaf color in various concentrations of colchicine and genotypes. Typically, the mutant of *Portulaca* leaves had an elliptical shape with pointed tips. However, the Peach genotype (G2) had differences in the shape and color of leaves in all colchicine concentrations (0%, 0.05%, 0.1%, 0.15%, and 0.2%) compared to other genotypes at the same concentration. These leaves were wilted with a reddish-green hue (Figure 4). The leaf changes were temporary and mainly occurred in the second week. The observed morphological changes represent variations induced by the colchicine treatment.

Flower color and shape

The color of the flower of *P. grandiflora* after colchicine treatment is presented in Figure 5. Figure 1 presents the tested genotypes before colchicine treatment. Changes in flower color are not yet visible in each genotype after colchicine application at various concentrations. Furthermore, there are no changes in the flower shapes of mutants of *P. grandiflora* in all treatment concentrations.

Correlation analysis

Figure 6 illustrates the interaction among the observed agromorphological traits of *P. grandiflora*. The leaf width strongly correlates positively with plant length (0.61). Strong positive correlations were also found for some characters, i.e., the number of branches on the shoot with the number of leaves (0.56) and the number of branches with the number of flowers (0.55). Statistical tests confirm the significance of the three correlation values (unmarked with 'X'), demonstrating a verified correlation between the observed traits.

Discussions

People commonly cultivate ornamental or medicinal plants in their yards. These plants have the dual function of beautifying the surroundings and being a source of alternative medicines. Additionally, there is an opportunity to generate additional revenue by trading these plants. *Portulaca grandiflora* Hook. is an ornamental and medicinal plant. The breeding program for *P. grandiflora* is primarily focused on increasing morphological diversity and biochemical content (Nurcholis et al. 2023). Mutation induction is crucial in enhancing genetic diversity in various cultivated plants. Induction of mutation and manipulation of plant polyploidy manipulation could be achieved using colchicine. Artificial polyploidy induction is carried out to improve plant traits and enhance the production of functional compounds (Ajayi et al. 2014; Le et al. 2020; Mangena and Mushadu 2023). Polyploidy plants may increase and improve the plant's economic value (Susrama et al. 2022; Tossi et al. 2022; Mangena 2023; Mohammadi et al. 2023).

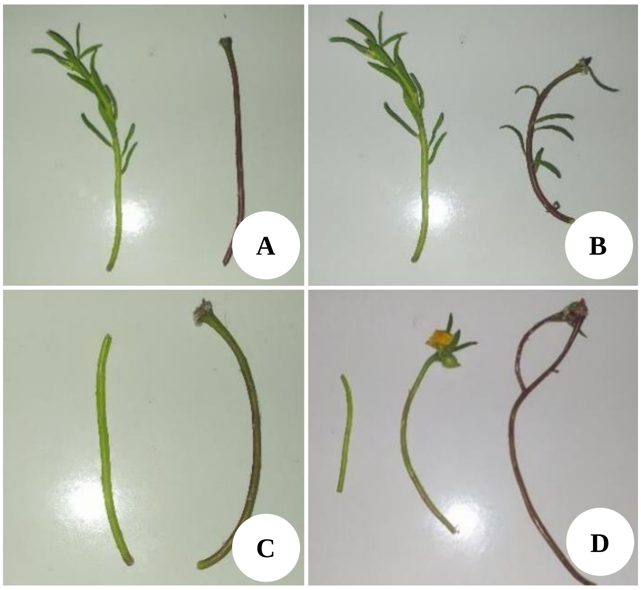

Figure 3. Stem color changes in *Portulaca grandiflora* plants: A. Orange genotype treated with colchicine 0.1%; B. Orange genotype treated with colchicine 0.2%; C. Peach genotype treated with colchicine 0.05%; D. Bicolor and Banana genotypes treated with colchicine 0.15%. The stem on the left side in each image represents the stem of the control plant, while the right represents the stem subjected to colchicine treatment

A B C D E F

Figure 4. Leaf shape and color changes in Peach genotype at all concentrations: A. 0%; B. 0.05%; C. 0.1%; D. 0.15%; E. 0.2%

Figure 5. Flower color and shape of tested genotypes of *Portulaca grandiflora* at week 12 after treatment: A. Orange genotype; B. Peach genotype; C. Bicolor genotype; D. Banana genotype

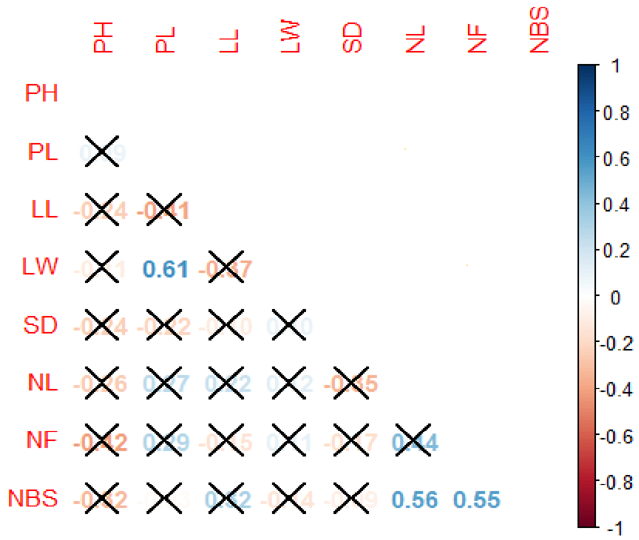

Figure 6. Correlation matrix of quantitative characters of *Portulaca grandiflora*. PH: Plant Height; PL: Plant Length; LL: Leaf Length; LW: Leaf Width; SD: Stem Diameter; NL: Number of Leaves; NF: Number of Flowers; NBS: Number of Branches on Shoot. 'X' marks in the cells indicate that the correlation value is not statistically significant (P<0.05)

Artificial mutation enhances the agromorphological traits of plants, particularly quantitative characteristics in various species, including *Vigna unguiculata* (L.) Walp. (Fathurrahman et al. 2023), *Praecitrullus fistulosus* (Stocks) Pangalo (Rasheed et al. 2022), *Zea mays* L. (Sinay and Tanrobak 2020), *Rhododendron fortunei* T.Moore ex Lindl. (Mo et al. 2020), and *Stevia rebaudiana* Bertoni) Bertoni (Grad and Gomaa 2020). In addition to enhancing agromorphological traits, colchicine treatment has successfully increased secondary metabolite content in plants (Ajayi et al. 2014; Le et al. 2020; Mangena and Mushadu 2023). Artificial mutation induction in *P. grandiflora* has been conducted using gamma irradiation (Aisyah et al. 2022) and colchicine (Sari et al. 2017a). Physical and chemical mutagens can randomly produce potential mutants with unique morphological changes.

The results of the study showed that the 'Banana' and 'Bicolor' genotypes are more responsive to colchicine application than the 'Peach' and 'Orange genotypes' The four genotypes showed different responses to colchicine treatment, indicating differences in tissue sensitivity to the mutagenic agent. Research by Sari et al. (2017b) reported that two varieties of *Coleus* have different LC_{50} values, indicating variations in tissue sensitivity among varieties to mutagen treatment. The level of tissue sensitivity in plants is often determined by evaluating the LC_{50} value. LC_{50} values for several crops using colchicine have been defined previously (Nandhini et al. 2019; Roy et al. 2023; Sodiq et al. 2023).

Further research must be performed to determine the specific LC50 concentrations for each genotype. Internal plant factors that cause differences in sensitivity to mutagens are nuclear index, ploidy level, and genetic background (mainly the DNA repair system) (Shu et al. 2011; Duarte et al. 2023). Additionally, technical factors can cause differences in sensitivity, including the type of mutagen, mutagen concentration, length of treatment, plant organs, water content, and oxygen (Oladosu et al. 2016; Muhammad et al. 2021; Chen et al. 2023). Induced polyploidy using colchicine was carried out on four genotypes of *P. grandiflora*, resulting in differences in size and the number of organs in *P. grandiflora* plants. The increase in DNA copies from the normal condition, resulting in cell enlargement, is called the 'gigas effect' (Manzoor et al. 2019). The 'gigas effect' is a phenomenon that underlies the increase in organ size observed in mutants of *P. grandiflora*. Niazian dan Nalousi (2020) reported that inducing mutagen, soaking time, and mutagen concentration are several factors for the success of polyploidy induction. Fathurrahman et al. (2023) state that applying colchicine for a prolonged time inhibits plant growth. Therefore, it is crucial to determine the concentration and duration of colchicine application in *P. grandiflora* to achieve the desired polyploidy level. Comparing the effectiveness among other polyploidy-inducing mutagens is interesting, considering both mutation frequency and spectrum. A study by Zhang et al. (2020) suggests that oryzalin is more effective than colchicine in manipulating polyploidy in *Caladium* and *Capsicum frutescens* L. (Pliankong et al. 2017). Colchicine and oryzalin are classified as antimicrotubule compounds (Pan et al. 2019). Antimicrotubule compounds disrupt microtubule integrity by binding to tubulin (Touchell et al. 2020). They influence cell division by hindering alpha and beta tubulin polymerization and preventing the format spindle fiber formation (Petersen et al. 2003). The treatment of colchicine did not change the shape and color of the flowers, but it affected the color of the stem and the color and shape of the leaves (Figures 3 and 4). The new variations and characteristics changes resulting from colchicine treatment could be a potential germplasm that may be further developed into new cultivars. However, the confirmation and validation of agromorphological traits in potential genotypes arising from induced mutations need further testing in molecular changes and subsequent generations. This is to ensure that changes can be inherited stably in each generation.

In conclusion, genotypes and colchicine treatment in *P. grandiflora* plants caused various morphological responses. Colchicine, a chemical mutagen, inhibits the growth and development of plants. Its usage can introduce plant diversity and enhance quantitative and qualitative plant traits. Treatment of 0.2% colchicine had the best response in the stem diameter of the banana genotype. Treatment of 0.05% colchicine resulted in the best response to plant height. The Orange genotype treated with 0.2% colchicine exhibited the best results for the number of flowers, while the number of branches on shoots was at a concentration of 0.05%. Several treatments change stem color, leaf shape, and leaf color. The treatment of the Orange genotype and a colchicine concentration of 0.2% is the most effective for enhancing the flowering of *P. grandiflora*. No significant differences were observed in flower shape and color. It is suggested that the dose range be expanded for further research to obtain changes in flower color, shape, and LC50 value.

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