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CHAMOMILE (*MATRICARIA CHAMOMILLA* L.) EXPLORES AGRO-MORPHOLOGICAL AND GENETIC VARIATION AFFECTED BY CHEMICAL MUTAGEN

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ABSTRACT

Chamomile (*Matricaria chamomilla* L.) is one of the most important medicinal plants in cosmetics and sanitary industries, with various applications. This research was carried out with a view to elucidate the efficacy of six doses of colchicine (0,025, 0.05, 0.1, 0.2, 0.4 and 0.8%) to improvement yield and enhance genetic diversity of chamomile mediated evaluate agronomical, chemical and molecular characteristics. Mutants with substantially superior economical properties, including number of branches, number of flowers, flower fresh and dry weight and the essential oil content, were all induced in colchicine treatments. The RAPD-PCR study has shown that the inducing mutants have been divided into two clusters. The genetic diversity coefficient has been measured at 30 and 35%. The main of two-stage polymorphism was 67.6%, and the Polymorphism Information Content (PIC) ranged between 0 to 0.449 in the bud stage and from 0 to 0.338 from in the seedling stage. Estimation of genetic diversity and selection of prefered mutants would be considerable importance in enhancement breeding programs and to identify of genotypes with higher desirable traits for further multiplication and commercial production. The usefulness of molecular markers as a method to identify the colchicine effect and meaningfully detect useful mutants was demonstrated in a combination of molecular level findings with physiological and morphological results found during various colchicine treatments. The 0.05% colchicine concentration for effective breeding of chamomile mutation is recommended in the results.

Keywords: Chamomile, genetic diversity, RAPD-PCR, colchicine, genetic improvment

INTRODUCTION

Matricaria chamomilla L. (Asteraceae), also known as true chamomile or German chamomile (Germplasm Resources Information Network (GRIN) 2019), is a generally outcrossing and originally diploid (2n = 18) species native to Europe and West Asia, with natural populations found in a variety of temperate zones of the world **(Franke 2007; Ruzicka *et al*., 2021**). Chamomile is a valued highly economically impactful medicinal plant. Agronomic practices in the cultivation of this plant are thus constantly improved. Chamomile cultivation is widespread in many regions due to its high medical and industrial importance(**Kwiatkowski, 2015**; **Surmacz-Magdziak and Wisniewski 2009)** German chamomile's active principles are: coumarin; flavonoids; α-bisabolol, α-bisabolol oxide A and B, chamazulene, sesquiterpenes; spiroethers and other components such as tannins, anthemic acid, choline, polysaccharides and phytoestrogens **(Karbalay-Doustb *et al*., 2010; McKay and Blumberg 2006**). Unfortunately, there are drastically limited genotypes in chamomile and the majority of chamomile varieties are landraces or merely populations (**Okoń *et al*., 2013**; **Otto *et al*., 2015**). Because of this the desire to find new outlets of diversity is important. Mutation breeding was actively conducted for crop breeding by increased genetic diversity and new character induction (**Bhor *et al*., 2014**; **Kharkwal and Shu 2009**; **Kishori Lal *et al*., 2019)** Colchicine is known as a chemical mutagenic agent that prevents the formation of microtubules from forming and resulting in mutagenic effects. This has been used on many ornamental plants to induce beneficial mutations. The mutagenic effects of colchicine on plant production have been demonstrated in numerous studies (**Castro *et al*., 2003**). Mutants have typically shorter stems and more fruit number (**Pickens *et al*., 2006**). The use of different levels of colchicine had a significant effect on content of essential oil of medicinal plants; for example in dragonhead (**Omidbaigi *et al*., 2010**), sesame **(Nura *et al*., 2013**), *Echinacea purpure,* (**Abdoli *et al*., 2013)**, *Salvia leriifolia* **(Estaji *et al*., 2017**), *Trachyspermum ammi* **(Sadat *et al*., 2017**). The diversity of mitochondrial (mt) has been studied to establish point mutations in different cultivated of chamomilla genotypes. 89 single nucleotide polymorphisms (SNP) have been identified from 33 genotypes **(Ruzicka and Novak 2020**). The use of molecular analyses is crucial for the accurate and fast identification of medicinal plants, as it is difficult to identify plant fragments sold on the market using conventional methods, especially due to the lack of morphological features (**Sánchez *et al*., 2020**). The combination of molecular markers and agronomic characteristics offers an excellent tool for indirectly selecting a trait of interest in the population (**Safaei-Chaeikar and Rahimi 2017**).
The purpose of this research was to illustrate how the efficacy of different colchicine concentrations were inducing new genotypes of chamomile, measuring the genetic diversity of the induced mutants, and evaluating the agronomic and yield components of these mutants.

MATERIALS AND METHODS

Vegetable Material

Farmer of Aromatic and Medicinal Plants, Beni Suef Governate, Egypt, kindly received seeds of a local accession of German chamomile, *Matricaria chamomilla*. Field experiments were conducted at a private farm in Beni Suef Governorate for three consecutive seasons (2015/2016, 2016/2017 and 2017/2018).

Colchicine treatments

Seedling treatments

Seeds were sown in plastic trays with peat moss and incubated in nursery. The grown seedlings were individually transplanted in plastic bags filled with claying soil 45 days later. These seedlings were treated with different concentrations of colchicine (0.0, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8%). Samples of the M_1-plants were obtained from each treatment. Furthermore, M_1-generation (first season) seeds were replanted for M_2-generation next year. Similarly, seeds from the M_2-generatin (second season) have been replanted for the M_3 generation.

Bud treatments

Following the stability of pot marigold plants (80 days from sowing) small cotton swabs soaked in aqueous colchicine solution at the same concentrations (0.0, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8%) were put over the first tight flower bud of plants and let dry entirely. Each treatment involved 30 plants (three replications each include10 plants).

Characters of vegetative growth

Plant height, number of branches/plant, number of flowers/plant, fresh and dry weight of flowers/plant (g) were all evaluated in three generations of mutants.

Chemical constituents

Total carbohydrates
The phenol-sulphoric acid method used to calorimetrically evaluate total carbohydrates (**Duboi *et al*., 1956**).

Essential oil

Using a Clevenger-type apparatus, essential chamomile oil from different treatments was achieved with 3 hours hydro-distillation. a Clevenger-type apparatus based on the method described in the current (**European Pharmacopoeia 2010**).

DNA extraction

The leaves were lyophilized by freeze-drying and then stored in -80°C freezer before use. Liquid nitrogen was used to grind the tissue samples into a powder. The genomic DNA was extracted using 0.1 g bulked tissue collected from individual plants from equivalent weights of freeze-dried leaf samples. The cetyltrimethyl ammonium bromide (CTAB) method was used to isolate DNA (**Hyam 1998; Dellaporta *et al*., 1983**). DNA extracted in TE buffer was re-suspended.

Confirmation of DNA quality

To determine the quality of DNA, as illustrated by (**Sambrook *et al*., 2006)**, samples of 5 µl from isolated DNA were tested on a 0.8 % agarose gel in the TAE buffer. DNA quality was determined and photographed using a UV-transilluminator.

Optimization of RAPD reaction

To standardize the PCR conditions, for amplification in the reaction using extract DNA, oligonucleotide primers (Table 1) was used. The reactions took place in a DNA Thermocycler. Each 25 *µl* reaction volume contained 12.5 *µl* Master Mix (one step PCR^{TM}), 2 *µl* of primer, 3 *µl* of genomic DNA (about 50 *ng*/µl) and 7.5 *µl* of deionized sterile water. The PCR reactions were performed under the following conditions: initial denaturation step at 94ºC for 5 min, then followed by 35 cycles of amplification at 94ºC for 1 min, 36ºC for 1 min, 72ºC for 1 min, followed by a final extension at 72ºC for 10 min were performed using thermal cycler 2720 (Applied Biosystems, USA). The PCR products were separated by 1.2% agarose gel prepared with embedded ethidium bromide by the 1X TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.3).

Table 1 The primers used and their nucleotide sequences.

No.	Primer code	Primers sequence (5'-3')
1	OPA-1	-5' CAGGCCCTTC 3'-
2	OPA-2	-5' TGCCGAGCTG 3'-
3	OPA-3	-5' AGTCAGCCAC 3'-
4	OPA-4	-5' AATCGGGCTG 3'-
5	OPA-5	-5' AGGGGTCTTG 3'-
6	OPA-6	-5' GCTCCCTGAC 3'-
7	OPA-7	-5' GAAACGGGTG 3'-
8	OPA-8	-5' GTGACGTAGG 3'-
9	OPA-9	-5' GGGTAACGCC 3'-
10	OPA-10	-5' GTGATCGCAG 3'-
11	OPC-1	-5' TTCGAGCCAG 3'-
12	OPC-2	-5' GTAAGGCGTC 3'-
13	OPC-3	-5 GGGGGTCTTT 3'-
14	OPC-6	-5' GAACGGACTC 3'-
15	OPC-7	-5' GTCCCGACGA 3'-
16	OPC-8	-5' TGGACCGCTG 3'-

Analyzing polymorphism

The genetic diversity of the DNA samples was evaluated using RAPD-PCR analysis. The reproducible was visually scored, polymorphic and monomorphic bands as present (1) or absent (0). In addition, faint reproducible RAPD bands were identified as present in the program of Numerical Taxonomy and Multivariate Analysis System Version 2.1 (NTSYSpc, ver. 2.1) (**Rohlf 2000**). The total number of bands per line was recorded for each primer, and the percentages of polymorphic band were calculated.

Statistical analysis

The experimental design used was randomized complete blocks with three replications, and the data was analysed using IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 24 (2016) for Windows.

RESULTS

Characters of vegetative growth

Table (2) indicates that the handling colchicine treated at all concentrations significantly decreased plant height. However, plant height is found to be inversely related to increasing colchicine concentration. It is also clear that colchicine treatment was less risky at the seedling stage than at the bud stage. Among plants handling with 0.8% colchicine at seedling and bud stages respectively, the shortest heights were (48.28 and 51.61 cm) as opposed to (67.70 cm) among control plants.

Table 2 Effect of colchicine on the vegetative growth characters; plant height, No. branches, No. flowers/plant, flower fresh and dry weight of *M. chamomilla* plant (mean of 3 seasons).

Treatments	plant height (cm)		No. branches		No. Flowers/ plant		flower fresh weight (g)		flower dry weight (g)	
Colchicine	Seedlings stage	Bud stage	Seedlings stage	Bud stage	Seedlings stage	Bud stage	Seedlings stage	Bud stage	Seedlings stage	Bud stage
Control	67.70 A	67.70 A	9.56 E	9.56 C	332.89 E	332.89 EF	3.65 E	3.65 D	0.74 E	0.74 C
0.03%	61.11 B	64.53 B	10.53 D	9.94 C	348.03 D	389.19 AB	3.93 D	3.71 D	0.81 D	0.76 C
0.05%	57.97 C	60.78 C	14.33 A	13.28 A	426.11 A	415.56 A	4.85 A	4.79 A	0.94 A	0.92 A
0.10%	54.47 D	56.53 D	12.78 B	12.5 AB	389.72 B	383.11 ABC	4.46 B	4.33 B	0.86 BC	0.84 B
0.20%	52.39 E	54.75 DE	12.56 B	11.63 AB	365.78 C	358.56 BCD	4.41 B	4.25 BC	0.89 B	0.86 B
0.40%	50.47 F	52.55 EF	11.61 C	11.03 BC	347.67 D	343.75 DEF	4.27 BC	4.1 C	0.83CD	0.82 B
0.80%	48.28 G	51.61 F	10.67 D	10.86 BC	334.31 E	308.58 F	4.17 C	4.09 C	0.84 CD	0.81 B
Mean	56.06 b	58.35 a	11.72 a	11.27 b	363.50 a	361.44 a	4.25 a	4.11 a	0.84 a	0.82 a

Legend: Means with different letter indicate significant difference between treatments p≤0.05

For the number of branches/plant, table (2) demonstrated a significant increase in the number of branches/plant at all concentrations of colchicine-treated plants. Nevertheless, it is clear that in this regard the medium dose (0.05%) was the most effective and that treating the plants at the seedling stage was more promoting than treating at the bud stage (14.33 and 13.28 respectively).

Number of flowers/plant as shown in Table (2) that treating chamomile plants with colchicine significantly increased number of flowers/plants. Especially the 0.05% concentration applied at both seedling and bud stages enhanced the maximum number (426.11 and 415.56) respectively.
Table (2), indicates that treatment of chamomile with the all concentrations of colchicine either in seedlings stage or in bud stage significantly increased the

fresh and dry weight of the flowers during the seasons of study. The moderate concentration (0.05%) of colchicine enhanced the highest significant records for both seedlings and bud stage dry weight (0.94 and 0.92 g respectively) and fresh weight (4.85 and 4.79) respectively.

Chemical constituents

Results tabulated in Table (3) show that treated plants with colchicine at (0.05%) increased total carbohydrates content compared with untreated plants, with the highest significant contents (13.05% and 12.82%) in seeding and bud stages respectively.

According to the data in Table (3), increasing colchicine is followed by increasing in essential oil content. Plants treated with colchicine at (0.05%) produced the highest significant content of essential oil (0.71 and 0.70 mg/g. f.w.) in seedling and bud stages, respectively, as compared to untreated plants.

Table 3 Effect of colchicine on carbohydrates and oil content of *Matricaria chamomilla* plant (mean of 3 seasons)

Treatments	Carbohydrates (%)		Oil content (mg/g f.w.)	
Colchicine	**Seedlings stage**	**Bud stage**	**Seedlings stage**	**Bud stage**
Control	11.77 CD	11.77 CD	0.57 BC	0.57 C
0.03%	11.76 CD	11.7 CD	0.57 BC	0.58 BC
0.05%	13.05 A	12.82 A	0.71 A	0.7 A
0.10%	12.62 AB	12.28 AB	0.62 B	0.62 B
0.20%	12.12 BC	12.03 BC	0.56 C	0.58 BC
0.40%	11.43 CD	11.55 CD	0.55 C	0.54 CD
0.80%	11.15 D	11.2 D	0.52 D	0.50 D
Mean	11.91 a	11.99 a	0.59 a	0.58 a

Legend: Means with different letter indicate significant difference between treatments $p \leq 0.05$

RAPD-PCR amplification

In the beginning, 16 random primers were tested for amplification of DNA from chamomile. 10 random decamer primers of OPA and OPC series namely OPA-01, OPA-02, OPA-03, OPA-07, OPA-08, OPA-09, OPA-10, OPC-02, OPC-07 and OPC-08 were successfully amplified and used in present studies. Individual DNA samples were subjected to primers, resulting in a total of 448 PCR reactions, and 10 µl of the PCR products ran on a 1.2 % agarose gel.

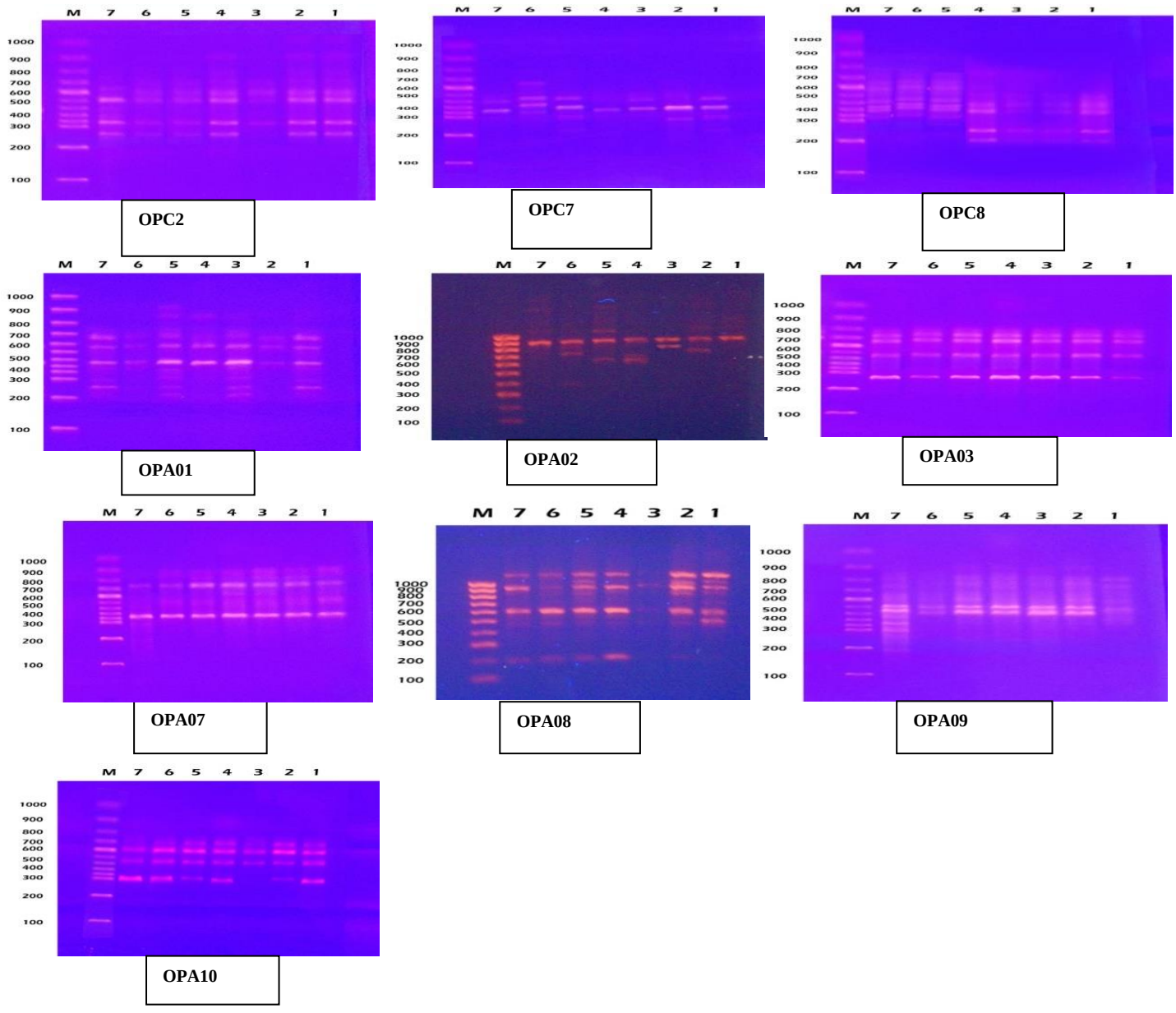

Figure 1 Profiles of RAPD primer amplified in *M. chamomilla* genotypes (seedling stage).
Legend: M (DNA ladder), and lanes from 1 - 7 represent 0.8%, 0.4%, 0.2%, 0.1%, 0.05%, 0.025% and 0% colchicine respectively.

Table 4 Characterization of selected RAPD primers with *M. chamomilla mutants*

Treatments	Seedling stage				Tight flowering bud stage			
Colchicine	Amplified products	Polymorphic product	% polymorphism	PIC value	Amplified products	Polymorphic product	% polymorphism	PIC value
OPC2	9	6	67	0.236	7	5	71	0.233
OPC7	8	7	88	0.286	8	8	100	0.388
OPC8	8	7	88	0.388	6	6	100	0.449
OPA1	8	5	63	0.255	7	4	57	0.209
OPA2	9	8	89	0.345	9	7	78	0.299
OPA3	4	0	0	0.00	4	0	0	0.00
OPA7	6	4	67	0.231	5	3	60	0.261
OPA8	8	6	75	0.245	9	5	56	0.172
OPA9	9	8	89	0.281	7	5	71	0.198
OPA10	5	3	60	0.179	4	3	75	0.326

The RAPD profile obtained (Fig. 1 and 2) illustrated a high level of polymorphism. The number of polymorphic products avaliable ranged from 3 to 8 per primer and the percentage of polymorphism 56 to 100%. The higher number of fragments yielded by one primer was 9 bands in the two stages (Table 4). Diversity analysis among chamomile genotypes and colchicine concentrations revealed higher diversity up to 35% indicating vast genetic variations among the genotypes can be detected Fig (3).

Assessment of genetic diversity and stability based on RAPD-PCR

The PCR results were used to evaluate the variation between the mutants that could be induced by the six concentrations of colchicine in seedlings and bud stages. Using RAPD technique, the resulting dendogram, following tests carried out and data processing was considered a true representation of the phylogenetic relationships between these mutants. In this study, genetic similarity matrix was used for cluster analysis using NTSYS pc software (Numerical Taxonomy and Multivariate Analysis System version 2.0).

Diversity among mutants induced by the different concentrations was high, about 34% and 36% in seedling and bud stages respectively; the control was separated in a cluster and the rest of concentrations in the second cluster Fig (3). There were two clusters, the first one include only the control, and the second cluster include the rest of mutants and it was divided into two sub clusters, concentration 0.005% in sub cluster and 0.0025, 0.1, 0.2, 0.4 and 0.8%in the second sub cluster Fig (3).

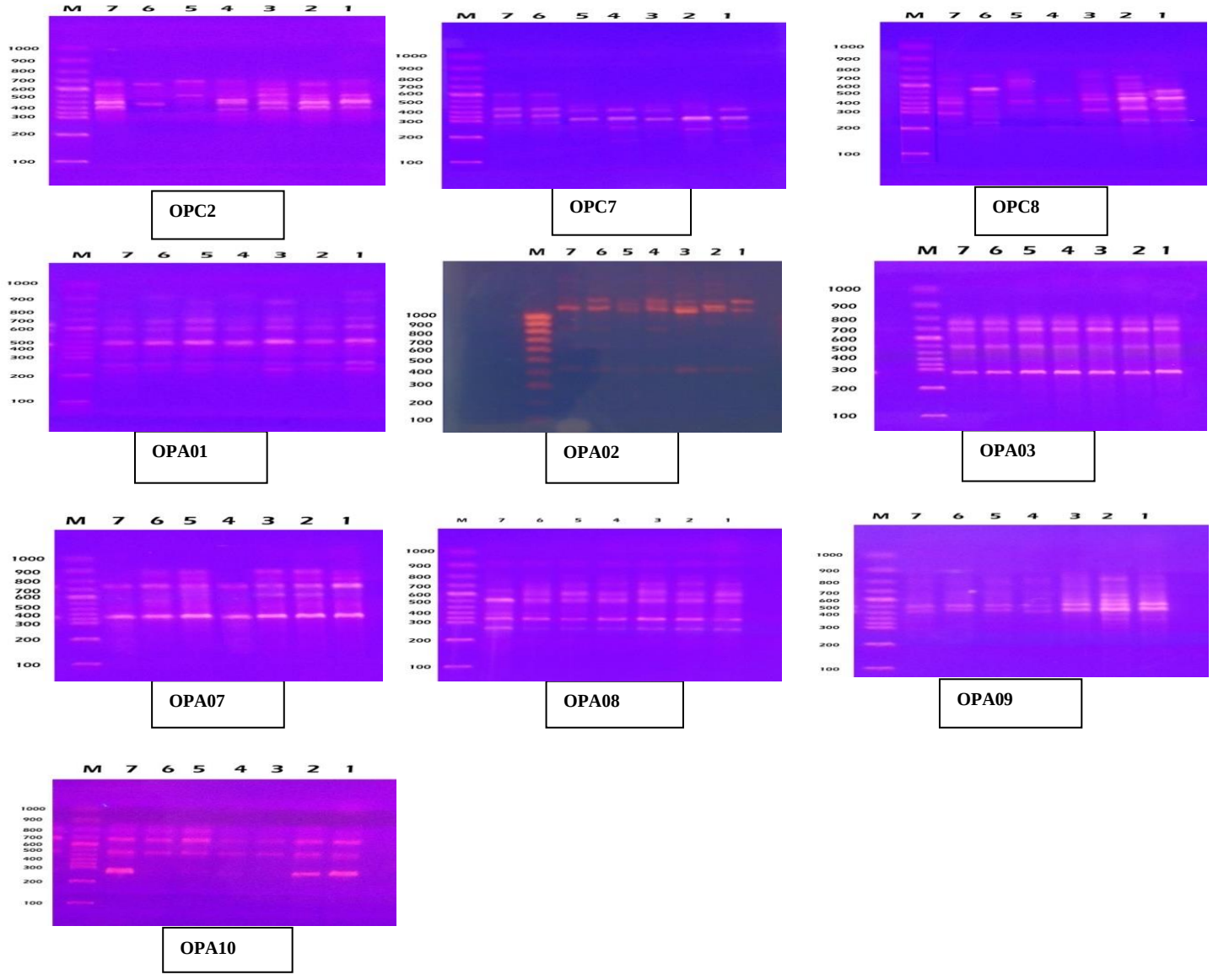

Figure 2 Profiles of RAPD primer amplified in *M. chamomilla* genotypes (tight flowering bud stage stage).
Legend: M (DNA ladder), and lanes from 1 - 7 represent 0.8%, 0.4%, 0.2%, 0.1%, 0.05%, 0.025% and 0% colchicine respectively.

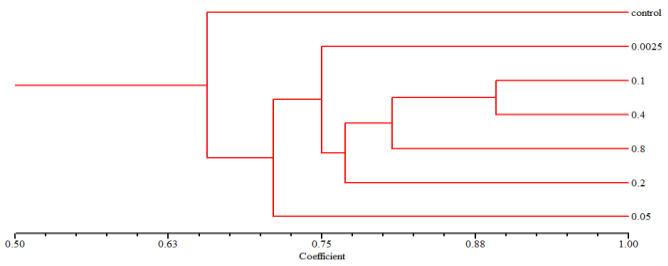

Fig (3a)

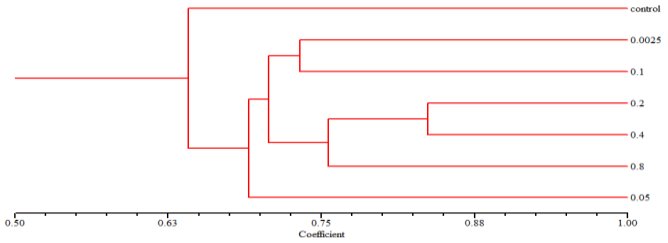

Fig (3b)

Figure 3 RAPD- based dendrogram of chamomile mutants induced by different concentration of colchicine applied at (a) seedling and (b) tight flowering bud stage.

DISCUSSION

Many authors reported similar findings on the decrease in plant height when caused by colchicine treatments for *Dracocephalum moldavica* (**Omidbaigi *et al.*, 2010**); (**Dheer *et al.*, 2014**) for Lablab purpureus; **(El-Nashar and Ammar 2016**) for *Calendula officinalis*; **(Estaji *et al.*, 2017**) for *Salvia leriifolia* and (**Kushwah *et al.*, 2018**) on *Chrysanthemum carinatum*. The decline of plant height may be due to the negative effect of colchicine on cells elongation, division and enlargement.

These results are in accordance with **(Nagahatenna and Peiris 2008**) on Hemidesmus indicus; **(Amiri *et al.*, 2010**) on *Datura stramonium*; (**Gantait *et al.*, 2011**) on *Gerbera jamesonii* and (**Nidhal *et al.*, 2013**) on *Trigonella foenum-graecum*. They reported that colchicine concentration was increase, the number of branches per plant increased. The high number of branches may be due to the influence of colchicine on activity of apical meristems for auxiliary branches of chamomile plant as well as enhancing its vegetative growth.

It seems that number of flowers is highly correlated with number of branches, this means that with increasing number of branches due to consuming more colchicine causes increasing number of flowers. This result could be attributed to the fact that chamomile plant is distinguished by high vigor growth especially branching ratio under moderate levels of colchicine. These results are in accordance with those obtained by (**Kobayashi *et al.*, 2008**) on *Salvia coccinea*; (**Majdi *et al.*, 2010**) on *Tanacetum parthenium*; (**Hannweg *et al.*, 2013**) on *Crocosmia aurea* and (***El-Nashar and Ammar 2016*)** on *Calendula officinalis*.

For fresh and dry weight of These results often resulted due to increasing cell size that in turn results in larger flowers on *Crocosmia aurea*, (**Zhang *et al.*, 2016**) on *Trollius chinensis*, (**Sadat *et al.*, 2017**) on *Trachyspermum ammi*, (**Wang *et al.*, 2017**) on *Fagopyrum tataricum* and (**Kushwah *et al.*, 2018**) on *Chrysanthemum carinatum*. They concluded that flowers fresh weight was increased by colchicine. These results are in agreement with those obtained by (**Hannweg *et al.*, 2013**) on *Crocosmia aurea*, (**Zhang *et al.*, 2016**) on *Trollius chinensis*, (**Sadat *et al.*, 2017**) on *Trachyspermum ammi*, (**Wang *et al.*, 2017**) on *Fagopyrum tataricum* and (**Kushwah *et al.*, 2018**) on *Chrysanthemum carinatum*. They indicated that colchicine treatment increased significantly or insignificantly all flowering parameter, as well as, flowering dry weight compared with control plants.

For the chemical constituents, the results are in accordance with those obtained by (**Estaji *et al.*, 2017**) on *Salvia leriifolia* and (**Abdoli *et al.*, 2013**) on *Echinacea purpurea*. They noticed that colchicine treatment significantly increased total carbohydrates. Table (3) indicates also that there is no significant difference on total carbohydrates contents between two stages of colchicine application. This result confirmed by (**Omidbaigi *et al.*, 2010**) on *Dracocephalum moldavica*, (**Estaji *et al.*, 2017**) on *Salvia leriifolia* and (**Sadat *et al.*, 2017**) on *Trachyspermum ammi* plant. There was no significance between two stages of growth for colchicine application in three seasons of study.

Variants in ornamental plants have been detected using molecular markers. DNA markers like RAPD are useful option to assess genetic diversity. These markers detect genetic variations that can be used to identify and discriminate closely related genotypes, to assess phylogenetic relationships, for pedigree analysis and linkage mapping (**Mohapatra and Rout 2005**; **Mikhaĭlovskiĭ *et al.*, 2007**; **Hassan *et al.*, 2019**). Diversity analysis among chamomile genotypes and colchicine concentrations revealed higher diversity up to 35% indicating reliable genetic variations among the genotypes can be detected by RAPD technique. Similar observations were obtained in chamomile,(**Okoń *et al.*, 2013**; **Okoń *et al.*, 2014**). Further (**Wolff and Rijn 1993; Shirao *et al.*, 2013**; **Kameswari *et al.*, 2014)** found genetic variations in chrysanthemum and (**Hassan *et al.*, 2019**; **Wolff and Rijn 1993**) in Calendula using RAPD technique.

Regarding the results of dendrogram it can be observed diversification of treatments effect. Authors used RAPD technique to detect the alterations of DNA and calculate the similarity or diversity between genotypes, species. For example, (**Wolff and Rijn 1993**) studied genetic variation in chrysanthemum mediated using Random Amplified Polymorphic DNAs (RAPDs) markers. Based on the SNP analysis, the genetic distance between 0.011 and 0.851 could be established for 19 mitochondrial haplotypes of chamomile and estimate diversity between/among
accessions **(Ruzicka *et al.*, 2021**; **Ruzicka and Novak, 2020**).

They found that the high level of polymorphism of RAPD fragments was useful for cultivar identification, and the diversity among control and treated plants. (**Lee and Kim 2000**; **Lema-Rumińska *et al.*, 2004**) used RAPD markers to study genetic diversity and genetic relationship between wild and three cultivars of chrysanthemum. RAPD markers were used for characterization chrysanthemum radiomutants (**Lema-Rumińska *et al.*, 2004**). The efficacy of the RAPD method for DNA fingerprinting of the original cultivars Richmond and its new radiomutants was demonstrated by PCR analysis (**Lema-Rumińska *et al.*, 2004**). Characterization of chrysanthemum radiomutants by RAPD was also done to understand the extent of diversity and similarity (**Kumar *et al.*, 2005**). (**Kaul *et al.*, 1990**) analysed mutations in *Dendranthema grandiflora* cv. Snow Ball through in vitro mutagenesis by exposing to gamma radiations. RAPD analysis was used to detection of polymorphism and to evaluate genetic similarity among genotypes. DNA change caused by colchicine was reflected in changes in RAPD bands through changes in disappearance of bands and/or appearance of new bands in the exposed plant. The disappearance of bands may be due to different reasons such as point mutation, formation of pyrimidine dimers and complex chromosomal rearrangement. High number of disappeared bands was observed with 0.05% of colchicine. This suggests that colchicine at these concentrations is capable of inducing DNA alterations that resulted in band loss. The new bands appear only due alterations of mutations (**Atienzar *et al.*, 1999**; **Atienzar and Jha 2006).** The appearance and disappearance of bands in this study could be related to changes or mutation of DNA of the plant induced by colchicine. (**Zainudin *et al.*, 2014**) measured genetic diversity within mutants of *Jatropha curcas* by RAPD. The RAPD markers have the potential to demonstrated genetic differences up to species and cultivar level, as reported by various researchers in several ornamental crops; (**Matsumoto and Fukui 1996**) and (**Jan *et al.*, 1999**) in rose; (**Wolff and Rijn 1993**) in chrysanthemum; (**Kumar *et al.*, 2012**) in *Dendranthema grandiflor*, (**Iqbal *et al.*, 1995)** in rhododendron sp.; and (**Hassan *et al.*, 2019**) in calendula. (**El-Nashar and Ammar 2016**) reported that SRAP technique was employed to confirm the existence of genetic alteration of DNA patern as a result of the mutagen concentration of colchicine. (**Soubra *et al.*, 2018**) used SCoT markers to evaluate the genetic diversity of chamomile and illustrated significant diversity between treated plants with different concentrations of colchicine.

CONCLUSION

Genetic diversity play a significant role in genotypes classification. Crop breeding and improvement programs, as well as genotype selection for more suitable characteristics for further multiplication and commercial cultivation, measurement of genetic diversity and selection of superior genotypes will be of great interest. A combination of molecular results with morphological and physiological responses observed for most of the tested parameters in all colchicine treatments and demonstrated the efficacy of RAPD-PCR as a method for detecting the colchicine effect on chamomile and could considerably to identify valuable mutants for breeding programs, particularly to improve yield component. Findings recommend the advantage of moderate level, 0.05% colchicine for efficient chamomile mutation breeding.

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