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

Ahmed A. M. Yassein¹*, Abd Allah H. A. Hassan², Shoukry M. Selim³, Ebtsam M Abdel-Allah²

Address(es): Ahmed A. M. Yassein,
¹Fayoum University, Faculty of Agriculture, Genetics Department, 63514, Fayoum, Egypt.
²Fayoum University, Faculty of Agriculture, Horticulture Department, 63514, Egypt.

*Corresponding author: aay00@fayoum.edu.eg

ARTICLE INFO

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 preferred 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 improvement

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 (Kwitkowski, 2015; Sarmacz-Magdziak and Wisniewski 2009). German chamomile’s active principles are: coumarin; flavonoids; α-bisabolol, α-bisabolol oxide A and B, chamazulene, sesquiterpenes; spiroetethers 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 (Onidhibaigi et al., 2010), sesame (Nura et al., 2013), Echinacea purpurea, (Abdoli et al., 2013), Salvia leucophylla (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

Seeding 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 M₁-plants were obtained from each treatment. Furthermore, M₁-generation (first season) seeds were replanted for M₂-generation next year. Similarly, seeds from the M₂-generatin (second season) have been replanted for the M₃ 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).

Characteristics 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-sulphuric 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™), 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 94°C for 5 min, then followed by 35 cycles of amplification at 94°C for 1 min, 36°C for 1 min, 72°C for 1 min, followed by a final extension at 72°C for 10 min were performed using thermal cycle 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' CAGCGCCCTTC 3'         |
| 2   | OPA-2       | 5' TGGCCGAGCTG 3'         |
| 3   | OPA-3       | 5' AGTCGACCAC 3'          |
| 4   | OPA-4       | 5' AATCCGGGTCTG 3'        |
| 5   | OPA-5       | 5' AGGGGTCTCCTG 3'        |
| 6   | OPA-6       | 5' GCTTCCCTGAC 3'         |
| 7   | OPA-7       | 5' GAAACCGGTGT 3'         |
| 8   | OPA-8       | 5' GTGACGTAAGG 3'         |
| 9   | OPA-9       | 5' GGGTACCCGC 3'          |
| 10  | OPA-10      | 5' GGTATCCGCAG 3'         |
| 11  | OPC-1       | 5' TTCCGACCCAG 3'         |
| 12  | OPC-2       | 5' GAAGGCGCTC 3'          |
| 13  | OPC-3       | 5' GGGGGTCTTT 3'          |
| 14  | OPC-6       | 5' GAACCGGACTC 3'         |
| 15  | OPC-7       | 5' GTCCCCGAGCA 3'         |
| 16  | OPC-8       | 5' TGAGCGTGTCG 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 (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) |
|------------|------------------|--------------|-------------------|------------------------|----------------------|
| 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%      | 61.1 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 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 seedling 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.

| Treatments | Carbohydrates (%) | Oil content (mg/g f.w.) |
|------------|-------------------|------------------------|
|            | 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≤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 *Matricaria chamomilla* plant (mean of 3 seasons).

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.
The RAPD profile obtained (Fig. 1 and 2) illustrated a high level of polymorphism. The number of polymorphic products available 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).

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

| Treatments | Seedling stage | Tight flowering bud stage |
|------------|---------------|----------------------------|
|            | 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     |

**Figure 2** Profiles of RAPD primer amplified in M. chamomilla genotypes (tight flowering bud 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.
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 (Okoño et al., 2013; Okoño et al., 2014). Further (Wolff and Rijn 1993; Shirio et al., 2013; Kameswari et al., 2014) found genetic variations in chrysanthemum and (Hassan et al., 2013; 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 using Random Amplified Polymorphic DNAs (RAPD) 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 accesses (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-Rumínska 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-Rumínska 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-Rumínska 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 grandiflorus cv. Snow Ball through in vitro mutagenesis by exposing to gamma radiation. 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 diversity at specific level as well as a model to various regions; it was used for 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 pattern 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.

DISCUSSION

Many authors reported similar findings on the decrease in plant height when caused by colchicine treatments for Drosophyllum 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 catarinum. 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 Trigongella foemum-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, which 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 aurora 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 aurora, (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 catarinum. 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 aurora, (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 catarinum. 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 content between two stages of colchicine application. This result confirmed by (Omidbaigi et al., 2010) on Drosophyllum 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 (Mohaaptra and Rout 2005; Mikhailovski et al., 2007; Hassan et al., 2019). Diversity analysis among chamomile genotypes and
RAUSCH.) raw material. Zesz. Probl. Postępów Nauk Rol., 542, 23–31.
Wang, L. J., Sheng, M.Y., Wen, P.C., Du, J.Y. (2017). Morphological, physiological, cytological and phytochemical studies in diploid and colchicine-induced tetraploid plants of *Fagopyrum tataricum* (L.) Gaertn. Bot. Stud., 58. https://doi.org/10.1186/s40529-016-0157-3
Wolff, K., Peters-Van Rijn, J. (1993). Rapid detection of genetic variability in chrysanthemum (Dendran tzelev) using random primers. Heredity (Edinb.), 71, 335–341. https://doi.org/10.1038/hdy.1993.147
Zainudin, A., Maftuchah, Fitriani, H. (2014). Analysis of genetic diversity on mutants jatropha curcas using RAPD. *Energy Procedia*, 47, 1–6. https://doi.org/10.1016/j.egypro.2014.01.189
Zhang, Q., Zhang, F., Li, B., Zhang, L., Shi, H. (2016). Production of tetraploid plants of Trollius Chinensis Bunge induced by colchicine. *Czech J. Genet. Plant Breed.*, 52, 34–38. https://doi.org/10.17221/89/2015-CJGPR