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STUDY OF MITOTIC INDEX PERCENTAGE BY THE INFLUENCE OF DIFFERENT CONCENTRATIONS OF MUTAGENSIS (COLCHICINE) IN DIFFERENT TIME DURATION ON TWO VARIETIES OF *CREPIS CAPILLARIES VIA* TISSUE CULTURE TECHNIQUE.

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ABSTRACT

***These*experiments were conducted at Plant Tissue Culture Laboratory/ college of Agricultural Engineering science / University of Duhok during the period from January 2011 to May 2013. The objectives of this study were to study the effects of different concentrations colchicine on mitotic index of *Crepis capillaris* (without and with 2B chromosomes) through long term callus culture until regeneration of plants from the callus, to investigate the effectivity of different concentrations of these chemicals in mitotic index induction in long-term callus culture. In general high concentration of colchicine and *Crepis capillaries* with 2B chromosomehave significant increase in mitotic index level as compared with other treatments however there were no significant differences between the two time durations in all passages except at passage five which performed that the first time duration of shaking recorded higher rate of callus cell mitotic index.**

KEY WORD: Cytogenetic, Chromosome, Tissue culture, Colchicine.
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INTRODUCTION

The chromosomal stability of *Crepis capillaris* callus at the diplod level was confirmed under various experimental conditions during one year of callus culture (Maluaszynska, 1990 and Jones *et al.*, 2007). The B chromosomes differ for the A chromosomes (normal chromosomes) because they are not necessary for normal plant growth and development, do not pair with the A chromosomes, and are inherited in a non-mendelian inheritance mode (Bougourd and Jones, 1997 and Trifonova *et al.*, 2002). Ploidy manipulation offers some benefits for horticultural, pharmaceutical and agricultural improvement of plants. Rhododendron tetraploids induced by oryzalin had larger leaves, flowers and pollen as compared with diploids (Contreras *et al.*, 2007). Since chromosomes have already multiplied but cell division is arrested, polyploidy cells are created. However, colchicine is carcinogenic and generally less effective than herbicides with a similar mode of action such as the dinitoanilines oryzalin and trifluralin (Hansen *et al.*, 2000). One of the reasons for the mitotic indexing of species is to generate data which is important for breeding purposes. Some of such data are the chromosomal attributes. Chromosome details are best studied on cells with optimal chromosome contraction or condensation, i.e. metaphase cells. These are best studied using root tips as well as floral buds (Osuji and Owei 2010).

MATERIALS AND METHODS

Sterilization of the Equipments

All culture vessels and glassware such as beakers, cylinders, jars, test tubes, glass rods and Petri dishes used in these experiments were carefully washed with detergent, then two times with distilled water and then all these glassware, were autoclaved. Furthermore, forceps and scalpels were first washed and dried using tissue paper and then autoclaved before use. It must also be pointed out that all the equipments were carefully warped in aluminum foil before autoclaving.

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Selection of Media

TheMedium that used was Murashige and Skoog media (1962) which has been specially formulated for plant cell, tissue and organ culture. This medium was obtained from HiMedia Laboratories (www.himedialabs.com; info@himedialabs.com). The Medium did not contain sucrose and agar; hence, these components had to be added to the medium before use.

Media Preparation

An amount of 4.41 g of the powdered medium was dissolved in dionized water and other ingredients were added. The pH of the medium was adjusted to 5.7 using diluted HCl or NaOH. The medium was usually prepared in lots of 1000 ml containers and then poured into small sterile glasses at 30 ml of medium for each glass and then autoclaved at 121 ^{0}C under a pressure of 1 kg/cm^2 for 15 minutes and left in growth room to be used for the next day.

Plant Material and Explants Preparation

Source of the seeds

Seeds of *Crepis capillaris* were obtained from Herbiseed (for specialist seeds) (www.herbiseed.com; Technical@herbiseed.com).

Seeds of *Crepis capillaris* (2n=6) and (2n=6 +2B chromosome) were sterilized in a mixture of 5 ml of absolute alcohol + 5 ml of 3% of H_2O_2 for 5 minutes and then washed several times with sterilized distilled water. The sterilized seeds were cultured in jars containing 30 ml of Murashige and Skoog (1962) solidified basal medium free from growth regulators. The cultures were maintained in growth room at 24 ± 1ºC, humidity 60-70% and 16 hours photoperiod (white, natural fluoresced light). After 4-5 weeks, the roots of developed plantlets were fixed to determine the % of mitotic index with an area of 1cm^2 were cultured on MS media supplemented with 5.0 mgl^{-1} NAA +0.2 mgl^{-1} BA to obtain callus. The calli of both plants were subcultured every 4-5 weeks. At each passage, only healthy and well growing callus pieces were transferred on to a new medium.

Treatments of Callus:

Four concentrations of Colchicin (0.0, 0.1, 0.125 and 0.15 mM) were used and 10 replications were assigned for each treatment.

The callus at 3rd passage was treated with colchicine in liquid medium for five and ten days duration using shakers (at 90 rpm). A minisart filter (0.45 µm) was used for the sterilization of the treatments.

The treated calli were washed with sterilized distilled water 5 times, then cultured on MS medium which was supplemented with 5.0 mgl^{-1} NAA + 0.2 mgl^{-1} BA and subcultured every 4-5 weeks until passage 10. At passage 10, the obtained calli were divided into two groups; the first group was subcultured every 4-5 weeks on MS media with the same composition until the end of the protocol. The second group was cultured on regeneration MS media without growth regulators. At passage 12th, the calli were subcultured on ½ strength media without growth regulators for two passages.

At passage 14th, the obtained callus was treated with different concentrations of BA and NAA for obtaining organogenesis and plants using half and full MS media.

The treatments used were:

A- 3.0 mgl^{-1} BA +0.5 mgl^{-1} NAA. B- 2.0 mgl^{-1} BA +0.5 mgl^{-1} NAA.
C- 1.0 mgl^{-1} BA + 0.2 mgl^{-1} NAA. D- 1.0 mgl^{-1} BA +0.5 mgl^{-1} NAA.
E- 4.0 mgl^{-1} BA +0.5 mgl^{-1} NAA. F- 5.0 mgl^{-1} BA +0.5 mgl^{-1} NAA.
G- 6.0 mgl^{-1} BA +0.5 mgl^{-1} NAA. H- 3.0 mgl^{-1} BA +3.0 mgl^{-1} NAA.
I-3.0 mgl^{-1} BA +2.0 mgl^{-1} NAA. J- 3.0 mgl^{-1} BA +1.0 mgl^{-1} NAA.
K- 2.0 mgl^{-1} BA +0.5 mgl^{-1} NAA. L- 7.5 mgl^{-1} BA +1.25 mgl^{-1} NAA.
M- 5.0 mgl^{-1} BA +1.25 mgl^{-1} NAA. N- 2.5 mgl^{-1} BA +1.25 mgl^{-1} NAA.
N- 2.5 mgl^{-1} BA +1.25 mgl^{-1} NAA. O- 10.0 mgl^{-1} BA +0.5 mgl^{-1} NAA.

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P- 10 mgl^{-1} BA +1.25 mgl^{-1} NAA. Q-12.5 mgl^{-1} BA +1.25 mgl^{-1} NAA.
Q- 15 mgl^{-1} BA +1.25 mgl^{-1} NAA. R- 7.5 mgl^{-1} BA +0.75 mgl^{-1} NAA.
S- 7.5 mgl^{-1} BA +0.5 mgl^{-1} NAA. T- 7.5 mgl^{-1} BA +0.25 mgl^{-1} NAA.
U- 5.0 mgl^{-1} BA +0.5 mgl^{-1} GA3.

Both liquid and agar solidified media were used for rooting stage. In case of liquid medium, a filter paper bridge was prepared and insert into the culture tube in such a way that the two arms were dipping into the liquid medium and on which the explants were placed and remained on the above medium. Both media were supplemented with 0.5 mgl^{-1} NAA.

At passage 21th, the roots of developed plantlets were fixed as mentioned previously in order to determine the mitotic index percentage. Moreover, the calli were fixed after 7 days of each passage.

Preparation of the Treatments

Colchicine Preparation

An amount of 0.0399 g of colchicine powder was dissolved in 100 ml distilled water to obtain (1mM) and then four different concentrations (0.0, 0.10, 0.125 and 0.15 mM) were prepared from this stock solution by taking (0.0, 10, 12.5 and 15 ml) and completing them to 100 ml using sterilized liquid (MS) medium.

Mitotic Index calculated as followes:

$$\text{Mitotic Index} = \frac{\text{Total Number of dividing cells}}{\text{Total Number of cells observed}} \times 100$$ (Yadav and Yadav 2010).

RESULTS

Figure (1) shows the effect of time durations on the percentage of callus cell mitotic index of *Crepis capillaris* (without and with 2B chromosomes) after colchicine treatments from passage one to passage fifteen. It demonstrates that there were no significant differences between the two time durations (five and ten days) in all passages except at passage five which perform, that the first time durations of shaking (5 days) recorded higher rate of callus cell mitotic index as compared with the second time durations of shaking (ten days).

Figure (2) manifests the effects of *Crepis capillaris* (without and with 2B chromosomes) on the percentage of callus cell mitotic index after colchicine treatments from passage one to passage fifteen. As it is noticeable from this figure, the *Crepis capillaris* with 2B chromosomes significant effect on increasing the rate of callus cell mitotic index percentages in all passages as compared with those recorded from *Crepis capillaris* without B chromosome.

The effect of colchicine concentrations on the percentage of callus cell mitotic index of *Crepis capillaris* (without and with 2B chromosomes) from passage one to passage nine is shown in Figure (3). High significant percentage of callus cell mitotic index recorded by the control treatment in first, third and fifth passages (2.34, 4.22 and 3.53%) respectively, while in second passage both treatments, control and 0.150 mM high significant influence over other treatments for increasing callus cell mitotic index (3.71 and 3.78%) respectively. Data in passage four, seven and nine declared that application of 0.150 mM of colchicine increased the percentage of mitotic index of calli cell as compared with other treatment, which were (5.18, 2.32 and 1.99%) successively. Regarding passage six, the third treatment (0.125 mM colchicine significant influences over other treatments which reached (2.49%). Results of passage eight clarify that application of 0.10 mM of

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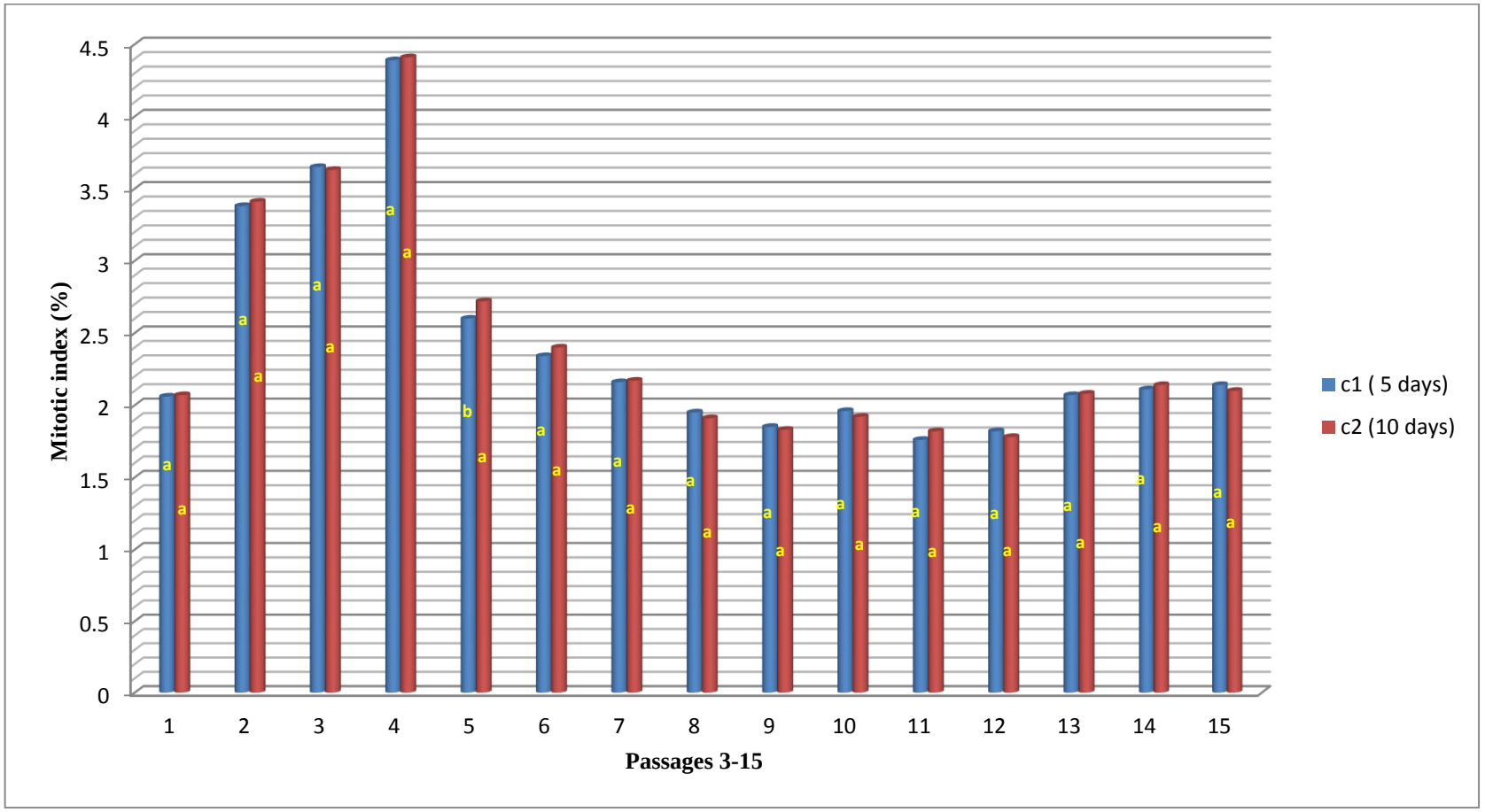

Fig.(1): Effect of Time Duration on thePercentage of Callus Cell Mitotic Index of *Crepiscapillaris* (without and with2B Chromosomes) after Colchicine Treatments from Passage 1 to Passage 15.

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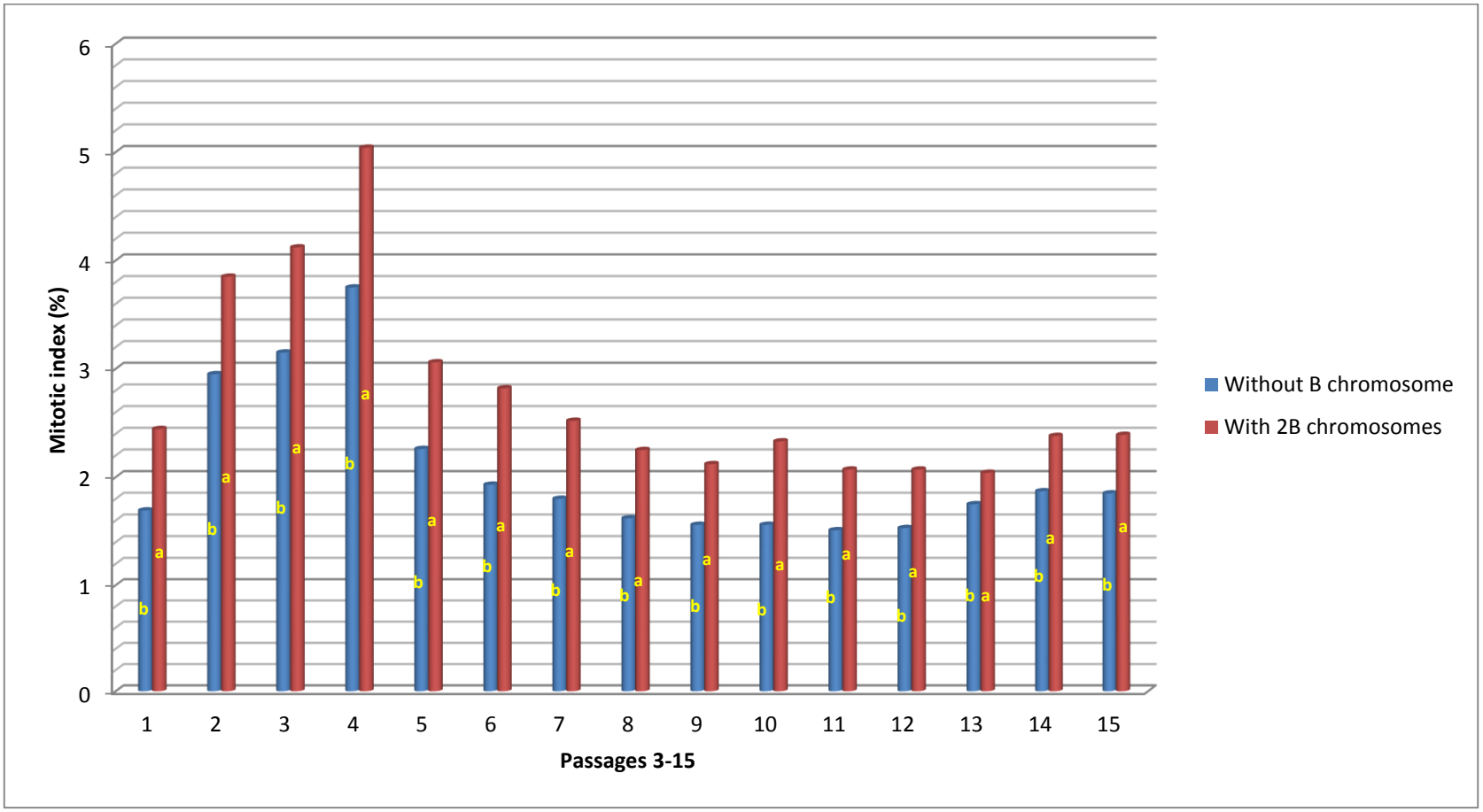

Fig.(2): Effect of *Crepiscapillaris*(without and with 2B chromosomes)on theCallusCellMitotic Index after Colchicine Treatments from Passage 1 to Passage 15.

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colchicine had a significant value for increasing callus cell MI which gained (2.09%) as compared with other treatments.

Figure (4) demonstrates, the effect of colchicine concentrations on the percentage of callus cell mitotic index of *Crepis capillaris* (without and with 2B chromosomes) from passage ten to passage fifteen. It is clearly perceived that in both passages, ten and twelve, both of the two treatments 0.100 and 0.125 mM of colchicine had a significant effect on increasing callus cell MI. Data in the passage eleven revealed that supplementation of 0.150 mM of colchicine inscribed high value for callus cell MI (2.17%) over other treatments. Concerning passage, fourteen and fifteen, the control treatment had the lowest value of mitotic index (1.91 and 1.83 %) respectively. High significant effect of callus cell MI was noticed in passage thirteen by 0.125 mM of colchicine which gave (2.27%).

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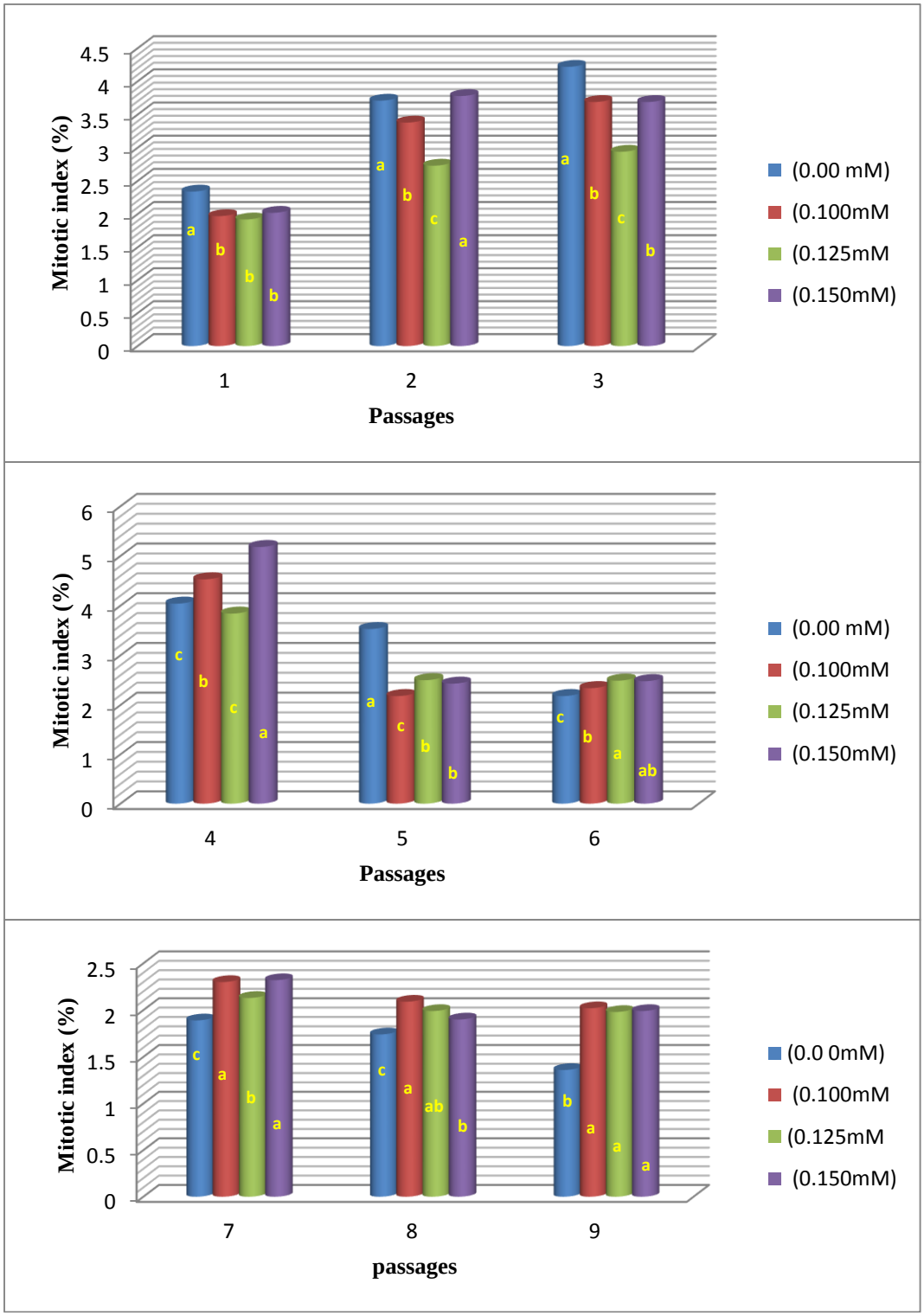

Fig.(3): Effect of Colchicine Concentrations on the Percentage of the Callus Cell Mitotic Index of *Crepis capillaris* (without and with 2B Chromosomes) from Passage 1 to Passage 9.

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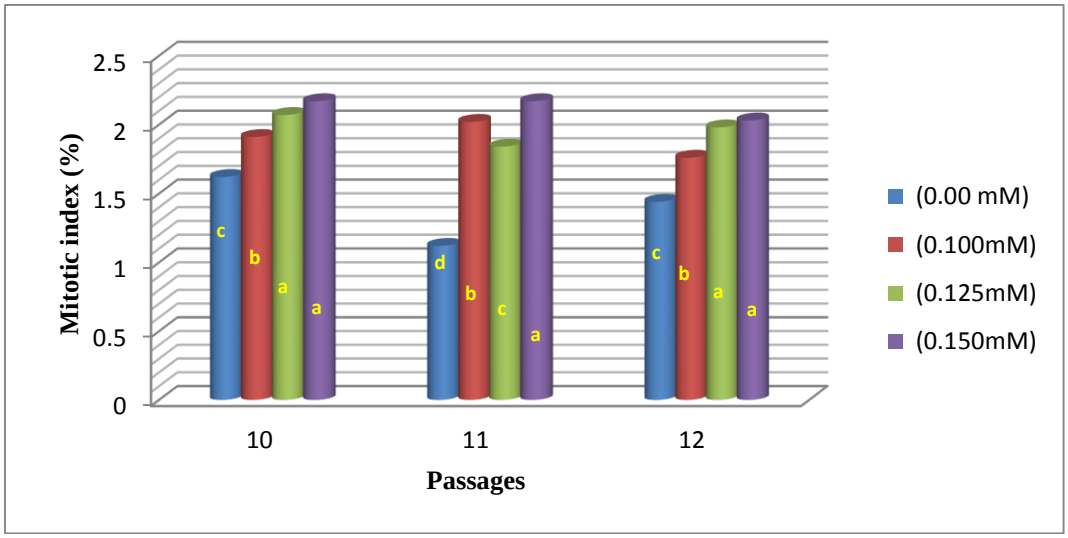

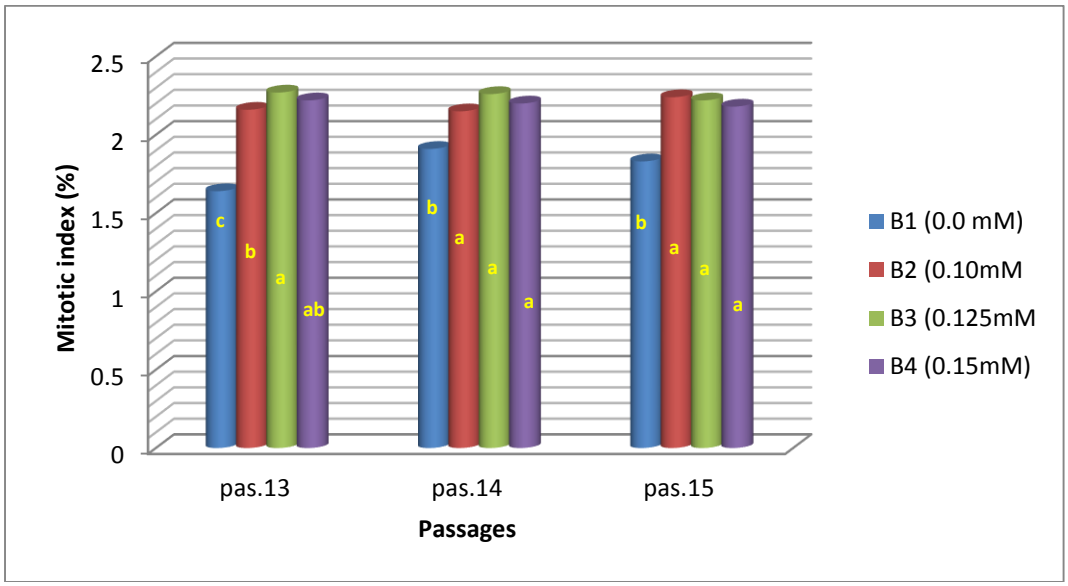

Fig. (4): Effect of Colchicine Concentrations on the Percentage of the Callus Cell Mitotic Index of *Crepis capillaris* (without and with 2B Chromosomes) from Passage 10 to Passage 15.

DISCUSSION

Figure (1) manifests that the there were no significant differences between the two time duration (five and ten days) on increasing MI (%) during all the passages except passage 5. Regarding Figure (2), it is clear that *Crepis capillaris* with 2B chromosomes had significant influences on increasing MI (%) over *Crepis capillaris* without B chromosome during fifteen passages. Figures (3 and 4) illustrate that higher application of colchicine (0.15 mM) increased MI (%) in most fifteen passages. The problem with respect to the effect of colchicine on mitosis was derived from two aspects:

a- The immediate effect of colchicines on the different phases of mitotic division.

b- Alteration in the mitotic cycle when the division starts in the colchicine containing secretion from the very beginning (Mehra, 1946).

As a long story of colchicine, Dermen (1940) stated the effect of colchicine on plant cell, colchicine like temperature, does not have any

effect on resting cells and like temperature its effect is specific to metaphase and telophase.

The immediate effect of colchicine on mitosis, preventing the anaphase separation of the chromatids (Levan, 1938), can be detected within 5 minutes of the beginning of treatment (Taylor, 1965). With a longer period of treatment, colchicine also leads to an increase in MI and in the number of cells in metaphase, a change in the prophase, metaphase ratio and the formation of polyploidy cells. The increase in MI within three hour may or may not be significant depending on the population of beans used (MacLeod, 1966), but it is highly significant after a 24 hrs of recovery period (MacLeod, 1965; Davidson and MacLeod, 1966) or after continuous treatment (Evans, *et al.*, 1957).

CONCLUSIONS

From the above results, it can be concluded that:

High concentrations of colchcine is effective in increasing Mitotic index % in *Crepis capillaris* with 2B chromosme then *Crepis capillaris* without B chromosome.*Crepis capillaris* without B chromosome was easily regenerated to plantlet, after treatment with colchcine and oryzalin in contrast to *Crepis capillaris* with 2B chromosomes.

RECOMMENDATIONS

A accordingly, it can be recommended to apply

Applying moleculer methods to follow up the changes in molecular level of *Crepis capillaris* with and without B chromosome after mutagen treatments and using other mutagens for chromosome doubling.

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 * A part of the second author's PhD dissertitation

خواندنا رێژا پێوانا کەرت کەرتبوونێ ب کاریگەریا رێژێن جوراوجور یێ جین گھورێ (کولچسین) ل دەمێن وەختی ییێن جیاواز لسەر دوو جورێن Crepis capillaries ب رێکا تەکنیکێن چاندنا شانا.

پوختە

ئەڤ ڤەکولینە ھاتە ئەنجام دان د لابورا چاندنا شانا/ کولیژا زانستێن ئەندازیاریا چاندنێ/ زانکویا دھوک ژ کانینا دووێ سالا 2011 ھەتا ئەیارا 2013. ئامانجا ئەڤێ ڤەکولینە زانینا کارتێکرنا رێژێن جوراوجور یێ کولچسینێ بوو لسەر پێوانا کەرت کەرتبوونێ یا Crepis capillaries (بێ و دگەل 2ب کروموسوم) ب درێژاھیا چاندنا کالسێ ھەتا نیکرنا رووەکێ ژ کالسێ، بۆ پشکنینا کارتێکرنا رێژێن جوراوجور یا ماددێن کیمیایی ل ھاندانا پێوانا کەرت کەرتبوونێ ب درێژاھیا چاندنا کالسێ. ب گشتی وێژا بلند یا کولچسینێ و Crepis capillaries دگەل 2ب کروموسوم، بلندبونەکا گرنگ ل ئاستێ پێوانا کەرت کەرتبوونێ وەك جیاوازی دگەل مامەلێن دی ھەرچەندە چ جیاوازییێن گرنگ د ناڤبەرا دوو دەمێن وەختی ل ھەمی بەشان دا نەبوو ژ بلی بەشێ پێنجێ کو ھاتیە ئەنجام دان ئەوێ ل دەما ئێکێ یا وەختی یا ھەژاندنێ بوو کو ھاتیە تومارکرن وەك بلندترین رێژە یا پێوانا کەرت کەرتبوونێ یێ خانا کالسێ.

دراسة النسبة المئوية للدلالة على مؤشر الانقسام عن طريق تأثير التركيزات المختلفة للگفرات (الكولجيسين) في فترة زمنية مختلفة على صنفين من *CREPIS CAPILLARIES* عبر تقنية زراعة الأنسجة.

الخلاصه

أجريت هذه التجربه في مختبر زراعة انسجة النبات / كلية علوم الهندسة الزراعية / جامعة دهوك خلال الفترة من يناير 2011 إلى مايو 2013. وكانت أهداف هذه الدراسة هي دراسة آثار تركيزات مختلفة الكولجيسين على مؤشر الانقسام ل*CREPIS CAPILLARIES*مع وبدون كروموسومات 2B من خلال استزراع الكالس على المدى الطويل حتى تجديد النباتات من الكالس ، للتحقق من فعالية التركيزات المختلفة من هذه المواد الكيميائية في تحريض مؤشر الانقسام في استزراع الكالس على المدى الطويل. بشكل عام ، فإن التركيز العالي من الكولجيسين *CREPIS CAPILLARIES*مع كروموسوم 2B له زيادة معنويه في مستوى مؤشر الانقسام مقارنة مع المعاملات الأخرى ، لكن لم تكن هناك فروق معنويه بين الفترتين الزمنيتين في جميع المراحل باستثناء في المرحله الخامسه الذي بينت بان الفتره الزمنيه الأولى من الهز سجلت ارتفاع فى معدل مؤشر الانقسام لخلية الكالس.

الكلمة الرئيسية: الوراثة الخلوية ، الكروموسوم ، زراعة الأنسجة ، الكولجيسين.

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