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Effect of magnesium chloride and Hydrogen peroxide on some active compounds Production in Phaseolus Vulgaris L. Using RAPD-PCR markers

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Abstract: The goal of the current research is to evaluate the effect of different chemicals on grown Phaseolus vulgaris L. plant, detect the effect of stimulants on the molecular characteristics responsible for the metabolism of secondary compounds, and detect and estimate mutations from RAPD-PCR marker reactions. Seeds were obtained from green bean plants. Research experiments were conducted for the three treatments with different concentrations. The seeds were planted on a medium of magnesium chloride salts (Mg1 = 200, Mg2 = 400 Mg/L). The treatment was hydrogen peroxide (P). The seeds were soaked at a concentration of 50% for a period of time. 4 minutes, control sample. After four weeks of cultivation, the concentration of the production of secondary metabolites Redaudioside and 3,4-Dimrthoxybenzyl was measured. The results of the concentration of the active compound Redaudioside showed the highest concentration in the (P) sample among the treatments, which reached 231.666 ppm, and the lowest value was in the Mg2 sample, which reached 70.461 ppm. Compared with the control sample, which amounted to 254.666 ppm, no plant stimulation occurred. As for the active compound 3,4-Dimrthoxybenzyl, the highest concentration was in the (P) sample, which reached 12.944, followed by the Mg1 sample, which reached 11,505 ppm, and the Mg2 sample was the lowest, reaching 0 ppm compared to the control sample, which reached 0 ppm. The results of the reactions of the RAPD-PCR indicators on the treatment of the studied materials showed that the total number of packages reached 163 packages, the number of general packages was 100 packages, and the number of mutant packages was 63 packages, distributed into 31 unique packages and 32 absent packages. The (Mg2) treatment was characterized by the highest number of unique packages, reaching 12 packages. Followed by Transaction (P) which amounted to 10 unique packages. Treatment (P) had the highest number of absent bundles, with 14 bundles, followed by treatment (Mg2), with 10 absent

bundles. As for the Mg1 sample, it gave the smallest number of unique and absent bands. We conclude from the results that the higher the concentration of magnesium chloride salts in the nutrient medium, the more delayed seed growth, the lower the measurement of the concentration of active compounds and the greater the number of genetic mutations, as the average concentration in magnesium chloride salts had the highest effect in terms of mutations according to the efficiency of RAPD-PCR reactions. As for hydrogen peroxide, it was highly stimulating and indicated the plant's response to high concentrations of secondary metabolites

Keywords: Phaseolus Vulgaris, peroxide Hydrogen, magnesium and RAPD-PCR markers

تأثير كلوريد المغنسيوم وبيروكسيد الهيدروجين في إنتاج بعض المركبات الفعالة لنبات Phaseolus vulgaris L. باستخدام مؤشرات RAPD-PCR.

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الملخص

هدف البحث الحالي هو تقييم تأثير المواد الكيميائية المختلفة، على نبات Phaseolus vulgaris L. المزروع نسيجياً والكشف عن تأثير المحفزات على الصفات الجزيئية المسؤولة عن ايض المركبات الثانوية، الكشف عن الطفرات من تفاعلات مؤشرات RAPD-PCR وتقديرها. تم الحصول على البذور من نبات الفاصوليا الخضراء، أجريت التجارب البحثية للمعاملات الثلاث بتراكيز مختلفة، زرعت البذور على وسط أملاح كلوريد المغنسيوم (Mg1 = 200، Mg2 = 400 Mg/L)، كانت المعاملة بيروكسيد الهيدروجين (P) نقعت البذور بتركيز 50% لمدة 4 دقائق، وعينة السيطرة. بعد اربعة أسابيع من الزراعة، تم قياس تركيز إنتاج مركبات الايض الثانوية Redaudioside و3,4-Dimrthoxybenzyl.بينت نتائج تركيز المركب الفعال Redaudioside اعلى تركيز في عينة (P) من بين المعاملات حيث بلغ 231.666 ppm وكانت اقل قيمة عند عينة Mg2 التي بلغت 70.461 ppm مقارنة مع عينة السيطرة التي بلغت 254.666ppm لم يحدث تحفيز للنبات. أما المركب الفعال 3,4-Dimrthoxybenzyl كانت أعلى تركيز عند عينة (P) الذي بلغ 12.944، بعدها عينة Mg1 التي بلغت 11.505 ppm وعينة Mg2 كانت الأقل بلغتppm 0 مقارنة مع عينة السيطرة بلغت 0ppm. بينت نتائج تفاعلات مؤشرات RAPD-PCR على معاملة المواد المدروسة، بلغ مجموع الحزم الكلي 163حزمة وعدد الحزم العامة 100حزمة وعدد الحزم الطافرة 63 حزمة توزعت على

31 حزمة فريدة و32 حزمة غائبة، تميزت معاملة (Mg2) اعلى عدد من الحزم الفريدة حيث بلغ 12 حزمة، تليها المعاملة (P) التي بلغت 10 حزم فريدة. أما المعاملة (P) إذ كانت أعلى عدد الحزم الغائبة بلغت 14 حزمة، تليها المعاملة (Mg2) بلغت 10 حزم غائبة. أما عينة Mg1 فقد اعطت اقل عدد من الحزم الفريدة والغائبة. نستنتج من خلال النتائج كلما ارتفع تركيز املاح كلوريد المغنسيوم في الوسط الغذائي كلما تأخر نمو البذور انخفض قياس تركيز المركبات الفعالة كلما زاد عدد الطفرات الوراثية حيث كان التركيز المتوسط في املاح كلوريد المغنسيوم هو الأعلى تأثيرا من ناحية الطفرات حسب كفاءة تفاعلات RAPD-PCR. اما بيروكسيد الهيدروجين كانت ذات تحفيز عالي وتدل على استجابة النبات للتراكيز العالية في مركبات الايض الثانوي.

الكلمات المفتاحية: الفاصوليا الخضراء، بيروكسيد الهيدروجين، المغنسيوم وعلامات RAPD-PCR

1. Introduction

Plant tissue culture technology is of great importance, as it allows the production of large numbers of plants that are completely similar in genetic composition to the mother plant and free of microbial contaminants from explants, callus induction, organogenesis, somatic embryogenesis and the production of active compounds [1]. One of the most important advantages of this technology is that it has been used in the field of improving plants' tolerance to stresses, and it can be grown in any season and within almost a short period, thus protecting plants from deterioration and extinction. It contributes to multiplying the genetic assets of plants and preserving them for a specific period of time, in addition to producing types of medicines and medicinal drugs [2,3]. The green bean plant, Phaseolus vulgaris L., is one of the important plants of the leguminous family that has been propagated through tissue culture, because it has nutritional, medicinal, and economic value. It is considered one of the most important food legumes used for human consumption due to its high content of protein, carbohydrates, vitamins, calcium, dietary fiber, and minerals that the body needs [4].

Magnesium chloride plays an important role in plant growth and development, acts as one of the components of chlorophyll molecules, and regulates the activity of key photosynthetic enzymes in chloroplasts [5]. Magnesium is one of the macronutrients that activates the work of enzymes and is the most important nutrient that the plant needs in small quantities. When used in small quantities, it is a stimulating factor for green bean plants, and when used in large quantities, it is a negative inhibitory factor on the plant [6]. Magnesium deficiency prevents plant growth and reduces crop production, and people need to nutritional supplements rich in magnesium to prevent deficiency of elements, and to obtain the optimal level of magnesium to prevent chronic diseases [7]. The harmful effects of salinity on plant growth result from osmotic stress and ionic toxicity observed in many regions of the

world. Magnesium chloride content in cultivated plants Sufficient availability of magnesium chloride generally ranges from 0.15 to 0.35%. The bean plant is affected by the high number of salts used in the nutritional medium, which affects the growth of parts of the plant such as roots, leaves, flowers, etc., and the plant becomes dormant [8].

Hydrogen peroxide, a weak acid, is a good bleach due to its strong oxidizing properties. Hydrogen peroxide is used as a sterilizer and catalyst at the same time in plant tissue culture. It belongs to the reactive group at the cell level. It plays an important role as the most stable type of oxygen in plants. There are some chemical signals that determine plant resistance to major biotic and abiotic stresses, called genetic expression, which are specialized genes for the defense system from external stimuli in the plant [9]. Treating green bean seeds with hydrogen peroxide helps them withstand drought. Activating antioxidants found in seeds and plants to mitigate the oxidative damage that leads to them, and improving the physiological characteristics of plant growth under drought. Drought is a major abiotic stress that affects agricultural systems and food production and induces numerous physiological, chemical, biological and molecular responses in many plants [10].

Using the technique of Random Amplified Polymorphic DNA (RAPD-PCR), which is characterized by ease and low cost, is the detection of genetic variations that depends on the presence or absence of duplicated bands in individuals. The use of molecular indicators to detect the genetic stability present within plant populations and determine the genetic relationships between Plant varieties and species [11]. The abundance of plant DNA fragments in random nucleotide sequences, where genetic material containing nucleotide sequences is amplified and can be seen in the form of bands of different molecular weights on an agarose gel. The basis of the work of this indicator depends on the use of short primers made of 10 nucleotides, and sometimes more, and the detection of many characteristics. Molecular analysis to assess genetic diversity [12].

2- METHODOLOGY:

2-1 Experiment Design:

The seeds were grown on MS nutrient medium, where the first treatment was to sterilize the seeds of Phaseolus Vulgaris L. with 50% hydrogen peroxide. As for the second treatment, a moderate amount of magnesium chloride salts is placed in the nutrient medium, and the seeds are planted on them and compared with the control sample as shown in Table (1). Four weeks after planting, parts of the treated cultivated plant are taken, such as young leaves and others, and the DNA is extracted from the plant and a procedure is performed. RAPD-PCR reactions in the plant tissue

culture laboratory and the molecular biology laboratory / College of Science / Tikrit University.

Table (1) shows the samples studied, the symbols, and the quantity used for each sample

Samples	Quantity	Symbols used
Control sample		C
hydrogen peroxide (H_2O_2)	50%	P
magnesium chloride salts	200	Mg1
magnesium chloride salts	400	Mg2

2-2 Sample collection:

Tissue samples were collected 30 days after culture under the influence of magnesium chloride salts and hydrogen peroxide. An amount of 3-4 new leaves were taken from the growing top, and the plant samples were transferred to the laboratory to isolate DNA from them and conduct reactions.

2-3 Extraction of active ingredients

The active substances are extracted using a Soxhlet device, and the examination is carried out using HPLC technology to measure the concentrations of the substances [13].

2-4 DNA extraction

DNA was extracted from young leaves using a CTAB method known as general methods [14].

2-5 Electrophoresis on agarose gel

Materials required for electrophoresis, such as SB solution, agar, red dye, and DNA samples, were prepared for electrophoresis.

2-6 Conduct RAPD_PCR reactions

RAPD-PCR reactions were performed for all studied samples of Phaseolus Vulgaris L., which express the change in the genetic stability of the plant, using 5 primers [15]. Table (2)

Table (2) shows the sequences of the RAPD_PCR primers used for the study

NO	Primers	Sequence 5´→→→ 3´
P 1	OPB-7	GGTGACGCAG
P2	OPC-3	GGGGGTCTTT
P3	OPC-4	CCGCATCTAC
P4	OPC-5	GATGACCGCC
P5	OPC-9	CTCACCGTCC

3- Method of extracting active compounds 3,4-Dimrthoxybenzyl, Redaudioside:

10 grams of vulgaris leaves were weighed and ground using a grinder. The powder is placed in a filter called a thimble and placed in a Soxhlet extraction device. Install the device in a beaker using (ethanol) as the solvent, i.e. adding 500 ml of ethanol. At a concentration of 99%, then the concentrate is installed and left for 24 hours to complete the soaking process and to give more room for extracting the active ingredient. The solvent is removed after extraction usually using a rotary evaporator at a temperature of 45-50°C [16].

4- The RAPD-PCR markers:

The RAPD-PCR master reaction mixture was prepared by mixing Premix (prepared by the company Pioneer) with 2 microliters of primer. 2 microliters of DNA were added to each sample with 10 microliters of Master Mix with 6 microliters of removed distilled water. ions, and the final volume of the mixture becomes 20 microliters, after which these tubes containing the mixture are placed in the polymerization device. The program mentioned in Table (3) is applied. After the reaction ends, the tubes are removed from the device and transferred or kept in freezer [17].

Table (3) solutions used in the RAPD markers

C	Components	Volume
1	Green Master mix	10µ l
2	Primer	2 µ l
3	Nuclease free water	6µ l
4	DNA template	2µ l
5	Total Volume	20µ l

First, 1 cycle of initial denaturation at 94°C for 4 minutes, then 40 cycles of denaturation at 93°C for 50 s, primer binding of 40 cycles of 36°C for 1 minute, elongation at 72°C and 40 cycles for 1 minute, Finally the final elongation for one cycle at 72°C for 10 minutes. After completing the amplification in the PCR machine, 4 µL of the PCR-RAPD products are taken and transferred to a 1.5% agarose gel with added DNA, and then the gel is photographed using ultraviolet light As in Table (4).

Table (4) RAPD-PCR reaction program

Stage name	temperature	the time	Number of courses
Primary metamorphosis	94°C	4 minutes	1
Secondary teratosis	93°C	50 s	40
Initiator link	36°C	1 minute	40
Elongation	72°C	1 minute	40
Final elongation	72°C	10 minutes	1

5- Diagnosis of mutations

The presence of mutations is detected through genetic variation in the DNA material that can be obtained from PCR and RAPD indicators and can be relied upon in determining the genetic differences of the samples compared to the control sample. The bands are placed in a table representing the number 1 for the presence of bands and the number 0 for the absence of bands [18].

5-1 Results of determining active compounds using an HPLC device

The results of Table (5) showed an increase in the control sample in the sugar compound Redaudioside of green bean plants compared to the compound 3,4-Dimrthoxybenzyl, which decreased significantly to 0 ppm. This indicates that green beans are more responsive to sugar compounds, especially to the compound Redaudioside, which reached 254.666 ppm.

Redaudioside compound stimulated green bean plants in most treatments, as the concentration of hydrogen peroxide P increased among all treatments, reaching 231,666 ppm. As for magnesium chloride salts, its value increased to 153.673 ppm in the medium treatment, and its value decreased to 70.461 ppm in Mg2, compared to the control sample. There was no change in the catalyst materials [19].

As for the compound 3,4-Dimrthoxybenzyl, which was more responsive when treated with magnesium chloride salts, it increased when adding low concentrations of Mg1, which reached 11.505 ppm, and decreased when adding high concentrations

of Mg2, which reached 0 ppm. Hydrogen peroxide Prose and reached 12.944 ppm. It is more responsive to green bean seed stimulation, which is considered to have good results on the plant and has high genetic variation and a change in morphological traits when compared with the control sample in the treatments.

Table (5) shows the catalysis of active compounds

	Samples	Redaudioside	3,4-Dimrthoxybenzyl
1	Control	254.666	0
2	hydrogen peroxide (P)	231.666	12.944
3	Mg1	153.673	11.505
4	Mg2	70.461	0

5-2 Results of RAPD-PCR indicators:

The results of RAPD-PCR showed that there is genetic variation at the DNA level in some samples, and all samples are characterized by containing varying mutations according to the effect of the plant-stimulating chemical. All samples were characterized by containing unique bands and absent bands, as shown in Tables (6), as it was shown that the results The primers used in the study for green bean plants: The number of sites produced was 67 sites, including 25 sites general to the treatments and the different sites produced by these primers. There are 42 sites, and the total number of packets is 163 packets. The number of general packets showed 100 packets, and the number of mutant packets was 63 packets resulting from the sum of unique packets and absent packets, which were 31 unique packets and 32 absent packets. The results of table (7) show the total of the variant packets produced in There were 63 treated samples, 31 of which were unique and 32 of which were absent. The hydrogen peroxide sample showed P. The number of unique bands was 10 and a number of Absent bundles 14 bundles. The sample of low magnesium chloride Mg1 had the least number of mutant bands, amounting to 9 unique bands and 8 absent bands. As for the second sample, Mg2, it had 12 unique bands and 10 absent bands. It was the most responsive green bean plant in terms of the number of unique bands among the treatments. It showed the highest value in the hydrogen peroxide sample P in terms of the number of mutant bands, 24 bands. The lowest number was 22 bands in the Mg2 sample, and the lowest number when treated with the Mg1 sample was 17 bands. This indicates the characteristics of the treatment that indicate the presence of unique bands. Absent bands at specific locations in the starter when the number of unique bands is more indicates the plant's response to the treated mutants.

Table (6) Results of the primers used in RAPD reactions for samples

Primer	P1	P2	P3	P4	P5	Total
Loci number	14	10	14	14	15	67
Public Locations	8	2	6	4	5	25
Differentiated locations	6	8	8	10	10	42
Total Packages	38	27	32	35	32	163
General packages	32	8	24	16	20	100
Mutant beams	8	11	8	24	12	63
Unique bands	1	7	8	11	4	31
Absent bands	7	4	0	13	8	32

Table (7) Results of the primers used in RAPD reactions for samples

Primer Number	Distinctive bands in Ants samples					
	P		Mg1		Mg2	
	Unique	Absent	unique	Absent	Unique	Absent
(OPB-7) P1	1	3	0	1	0	3
P2 (OPC-3)	2	1	3	2	2	1
(OPC-4) P3	3	0	2	0	3	0
(OPC-5) P4	3	6	4	4	4	3
(OPC-9) P5	1	4	0	1	3	3
Total	10	14	9	8	12	10
	24		17		22	
63						

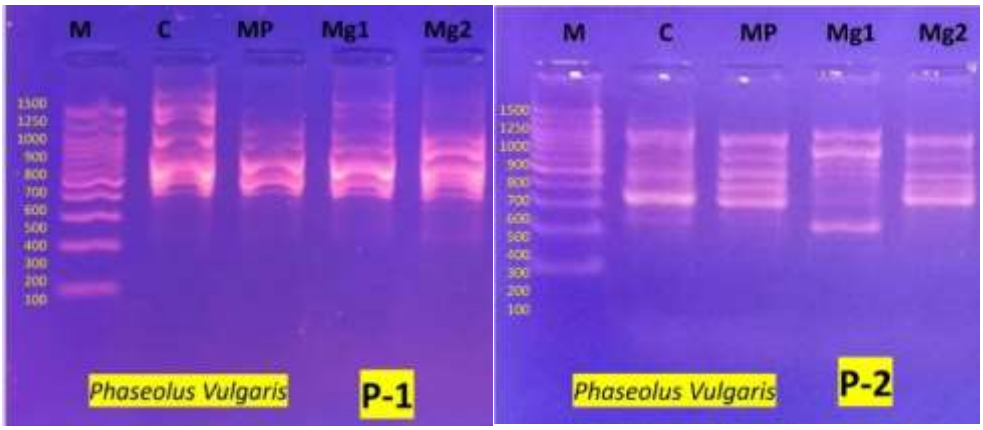
Discussion:

It was shown from the results that whenever we used medium concentrations of magnesium chloride salts in the MS nutrient medium, the speed of germination increased, and the concentrations of the active substances increased in the Mg1 treatment using two types of active compounds: Redaudioside and 3,4-Dimrthoxybenzyl, and decreased in the Mg2 treatment if it was the most responsive to compound 3. 4-Dimrthoxybenzyl indicates that the

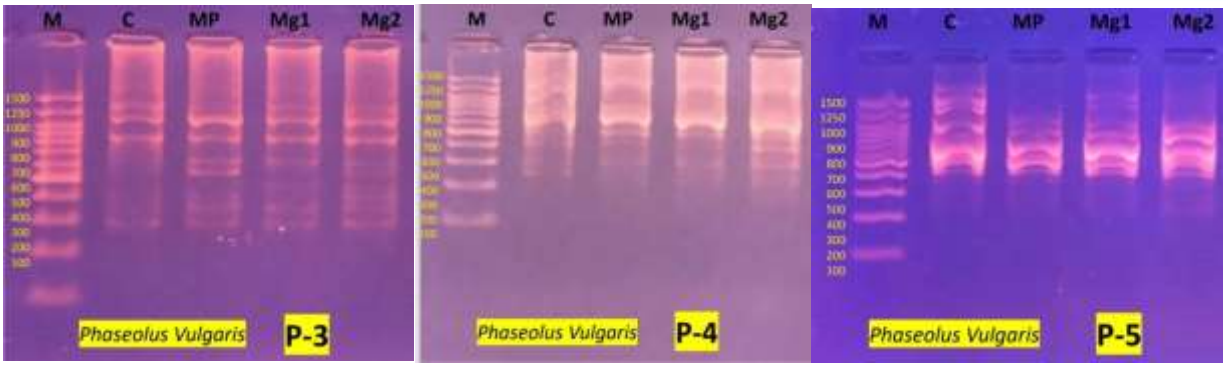
proportionality is inverse, as mentioned in previous studies (Bhardwaj, S., & Kumar, P.2020) It has been shown that one of the most important factors that help in the success of the plant tissue culture technique depends on the ability of the seeds to germinate and form strong-growing seedlings that can withstand salt stress and the environmental conditions that they face during their growth period. With the tissue culture technique, the absorption of magnesium chloride salts occurs directly. From the middle and quickly affects the external appearance of the plant, especially the roots and the appearance and vital characteristics of the plant. Excessive salinity from magnesium chloride causes a noticeable decline in the production of compounds. Effectiveness and genetic stability compared to the control sample, the green bean plant is one of the non-salinity-loving plants (Gupta *et al*., 2022). As for the results of hydrogen peroxide P, it appears that the more the concentrations used average 50%, the higher the concentration of the active substances and the faster the plant grows. The substances are considered Chemicals are among the sterilizing and stimulating factors at the same time, which express a change in genetic stability, which achieved good changes in some phenotypic traits and the production of a large number of active compounds in the plant due to the stimulation occurring in the seeds of the green bean plant, and it was the most stimulating factor. The 3,4-Dimrthoxybenzyl compound, when compared with the control sample, reached 0 (Alakhdar *et a*l., 2021). The stimulants on the plant are detected through HPCL technology to measure and concentrate the plant's active compounds and compare them with the control sample, as the value of the active compounds in the plant increases indicates the presence of an important factor in improving the plant's characteristics (Bettaiah *et al*., 2021). The mutations occurring in the genetic stability to which the plant was exposed are detected and compared with the control sample. The most influential in terms of the number of mutant bands of hydrogen peroxide P, which reached 24 bands, 10 were unique and 14 were absent, while the concentrations of magnesium chloride Mg2 were of medium concentrations and the smallest number of Mutations upon treatment with a small amount of magnesium chloride through the RAPD-PCR reaction technique, which is highly efficient and a quick and inexpensive way to detect these variations. Genetic studies obtained from the green bean genome to improve plant production in environments exposed to salinity (Al_sugmane *et al*, 2020).

Conclusion:

From an appearance perspective, salt stress has an inhibiting effect on the vegetative growth of the plant. The higher the concentration of salts, the more the seeds will be delayed in germinating as a result of obstructing the absorption of materials from the nutrient medium. This increase in salts causes stunting of the plant due to a defect in the physiological processes of the plant to resist the toxic effect of magnesium chloride ions, causing a deficiency. The result is the amount of active compounds produced by the plant, which has an important role in the type of activities that occur, which causes an inverse relationship. The higher the concentration of magnesium chloride, the lower the concentration. Active ingredients of green bean plant. As for hydrogen peroxide, there is a direct relationship between the concentration of hydrogen peroxide and the concentration of active substances. The more the concentration averages 50% and the shorter the seed exposure period, the faster the plant's response will be with a good number of active compounds. Through this, mutations occur in the genome of the Phaselus Vulgaris plant. Correlation relationship between measuring the concentration of active substances and molecular interactions. The results showed the concentration of active substances and molecular characteristics that there is a correlation relationship between them. The correlation relationship appeared in treatment P between the number of unique bands, the number of absent bands, and the number of active materials. The greater the amount of active materials, the greater the number of mutant bands in peroxide. Hydrogen P had the highest number of mutant bands at 24 among other treatments, and unique bands were less than absent. As for magnesium chloride salts, Mg2, with an average concentration, there was a direct relationship. The higher the amount of active ingredients, the more the number of bands, as the number of mutant bands reached 22, and the number of absent bands was less, at 10, compared to the lowest concentration of magnesium chloride at Mg1, which reached the lowest number. Mutant packages.



Picture (1,2): Primers used for Phaseolus Vulgaris



Picture (3,4,5): Primers used for Phaseolus Vulgaris

6-REFERENCES

1-Kim, D. H., Gopal, J., & Sivanesan, I. (2017). Nanomaterials in plant tissue culture: the disclosed and undisclosed. *RSC advances*, *7*(58), 36492-36505.

2- Chandran, H., Meena, M., Barupal, T., & Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnology reports, 26, e00450.

3- Didinger, C., Foster, M. T., Bunning, M., & Thompson, H. J. (2022). Nutrition and human health benefits of dry beans and other pulses. *Dry Beans and pulses: Production, processing, and Nutrition*, 481-504.

4- Bidabadi, S. S., & Jain, S. M. (2020). Cellular, molecular, and physiological aspects of in vitro plant regeneration. Plants, 9(6), 702.

5- Zhang, B.; Cakmak, I.; Feng, J.; Yu, C.; Chen, X.; Xie, D.; Wu, L.; Song, Z.; Cao, J.; He, Y. Magnesium deficiency reduced the yield and seed germination in wax gourd by affecting the carbohydrate translocation. *Front. Plant Sci.* 2020, *11*, 797. [Google Scholar] [CrossRef]

6- Liu, M., Yu, X., Li, M., Liao, N., Bi, A., Jiang, Y., ... & Zeng, W. (2018). Fluorescent probes for the detection of magnesium ions (Mg 2+): from design to application. *RSC advances*, *8*(23), 12573-12587.

7- Ciscomani-Larios, J. P., Sánchez-Chávez, E., Jacobo-Cuellar, J. L., Sáenz-Hidalgo, H. K., Orduño-Cruz, N., Cruz-Alvarez, O., & Ávila-Quezada, G. D. (2021). Biofortification efficiency with magnesium salts on the increase of bioactive compounds and antioxidant capacity in snap beans. *Ciência Rural*, *51*(6), e20200442.

8- Ishfaq, Muhammad, et al. (2022) "Physiological essence of magnesium in plants and its widespread deficiency in the farming system of China." Frontiers in plant science 13: 802274.

9- Hung, K. T., Hsu, Y. T., & Kao, C. H. (2006). Hydrogen peroxide is involved in methyl jasmone-induced senescence of rice leaves. Physiologia Plantarum, 127(2), 293-303.

10- Silva, P.C.; AzevedoNeto, A.D.; Gheyi, H.R.; Ribas, R.F.; Silva, C.R.; Cova, A.M. Seed priming with H_2O_2 improves photosynthetic efficiency and biomass production in sunflower plants under salt stress. *Arid. Land Res. Manag.* 2021, *5*, 1–5. [Google Scholar] [CrossRef]

11- AL-Sagiman Rafea Zaidan. Ali Muhammed Abed. Akeel H. Ali AL-Assie. (2021). The Scientific Effects of Different Sounds Volume on the phenotypic and Genetic Markers of Cucumissativus.ISSN:1583-6258, Vol. 25, Issue 3, Pages.7416-7430.

12- Ahmed, H. A., & Amein, K. A. (2023). Influence of Peanut Seed Exposure to Microwave and X-ray Radiations on Sclerotium Stem Rot and Effects on Yield Improvement, and RAPD-PCR Mutagenic Analysis of Peanut Plants. Egyptian Journal of Phytopathology, 51(2), 14-29.

13- Hussain A., Quadri I.A., Nazir H., Ullah I. (2012b) Plant tissue culture: current status and opportunities. In: Recent Advances in Plant In vitro Culture, Leva A., Rinaldi L.M.R., Eds., Intech open-access pp. 1-29.

14- Huang, Q.X.; Wang, X.C.; Kong, F.; Guo, Y.L. and Guo, A.P. (2013). An efficient DNA isolation method for tropical plant. Far. J. Bio, 12(19) :2727-2732.

15- Singh, K.P. and Roy D. 2004. Somatic mutations in stilbene estrogen-induced Syrian hamster kidney tumors identified by DNA fingerprinting. Journal of Carcinogenesis.

16-Alsoufi, M. S., & Elsayed, A. E. (2018). Surface roughness quality and dimensional accuracy—a comprehensive analysis of 100% infill printed parts fabricated by a personal/desktop cost-effective FDM 3D printer. Materials Sciences and Applications, 9(01), 11.

17-Kumar, N. S., & Guru Subramanian, G. (2011). Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci Vis*, *11*(3), 116-124.

18-Faezah Al-Sadat Mortazavi Moghadam, Ardeshir Qaderi., Gholam-Reza Sharifi. (2021). Evaluation of Genetic Diversity of 17 Populations (*Phaseolus Vulgaris* L.) Plant Collected from Different Regions of Iran by RAPD Marker.

19- Prakash, I., Markosyan, A., & Bunders, C. (2014). Development of next generation stevia sweetener: Redaudioside M. *Foods*, *3*(1), 162-175.

20- Bhardwaj, S., & Kumar, P. (2020). Salinity stress, its physiological response and mitigating effects of microbial bio inoculants and organic compounds. *Journal of Pharmacognosy and Phytochemistry*, *9*(4), 1297-1303.

21- Gupta, A., Mishra, R., Rai, S., Bano, A., Pathak, N., Fujita, M., ... & Hasanuzzaman, M. (2022). Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. International Journal of Molecular Sciences, 23(7), 3741.

22- Alakhdar, H. H., & Shoala, T. (2021). Exogenous application of hydrogen peroxide in different resistant bean cultivars of Phaseolus vulgaris to Tetranychids urticate (Acari: Tetranychidae). *Arthropod-Plant Interactions*, *15*(3), 439-445.

23- Bettaiah, A., & Prabhushankar, H. B. (2021). Screening of novel source for genistein by rapid and sensitive UPLC-APCI-TOF mass spectrometry. *International Journal of Food Science*, *2021*(1), 5537917.