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Comparative Study of the Effect of Different Concentrations of Salinity on the Morphological and Molecular Characteristics of *Lepidium sativum* L. Using RAPD-PCR Markers

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ABSTRACT

This study aimed to evaluate the effects of different salinity levels on the morphological and genetic traits of *Lepidium sativum* L. (garden cress). Three treatments using well water with varying salinity levels (5.30, 19.17, and 23.35 mg/L) were compared to a control irrigated with Tigris River water (0.80 mg/L). Parameters measured included germination time, plant height, number of leaves and branches, and chlorophyll content. Germination occurred first in M1 (18 hours), followed by M2 (24 hours), and M3 (72 hours), indicating an inverse relationship between salinity and germination speed. M1 plants reached the greatest height (30.67 cm), followed by M2 (30.02 cm), and M3 (16.28 cm). Leaf count was highest in M1 (23), then M2 (22), and lowest in M3 (10). Branch count peaked in M2 (7.1), followed by M1 (6.7), with M3 lowest (5.5). Chlorophyll content was highest in M2 (38.14 Soil Plant Analysis Development (SPAD)), then M1 (37.76 SPAD), and lowest in M3 (33.62 SPAD). Randomly Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR) analysis was conducted to assess genetic variation. A total of 135 bands were observed: 83 were absent and 52 unique. M2 showed the highest number of unique bands (20), followed by M3 (18), and M1 (14). For absent bands, M2 had 32, M1 had 29, and M3 had 22. The results demonstrate that increased salinity negatively affects germination, plant growth, and genetic diversity, with moderate salinity inducing more genetic variation than high or low levels.

Keywords: Genetic markers, *Lepidium sativum* L., Mutations, RAPD-PCR, Salinity.

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دراسة مقارنة لتأثير تراكيز مختلفة من الملوحة على الصفات المظهرية والجزيئية لنبات الرشاد *Lepidium sativum* L باستخدام مؤشرات RAPD-PCR

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الملخص

هدفت هذه الدراسة إلى تقييم تأثير مستويات مختلفة من الملوحة على الصفات المورفولوجية والجينية لنبات الرشاد (*Lepidium sativum* L.). تم استخدام ثلاث معاملات بمياه آبار ذات مستويات ملوحة متفاوتة (5.30, 19.17, & 23.35 mg/L) ومقارنتها بعينة سيطرة رُويت بماء نهر دجلة (0.80 mg/L). شملت القياسات وقت الإنبات، طول النبات، عدد الأوراق والفروع، ومحتوى الكلوروفيل. بدأ الإنبات أولاً في M1بعد 18 ساعة، تلاه M2 بعد 24 ساعة، وM3 بعد 72 ساعة، مما يشير إلى علاقة عكسية بين تركيز الملوحة وسرعة الإنبات. سجلت نباتات M1 أعلى طول (30.67 cm)، تلتها M2 (30.02 cm)، وكانت M3 الأقل (16.28 cm). كان عدد الأوراق الأعلى في M1 (23 ورقة)، ثم(22) M2 ، والأقل (10) M3. وبلغ أعلى عدد فروع في M2 (7.1)، تلتها(6.7) M1 ، بينما كانت M3 الأدنى (5.5). وكان أعلى محتوى من الكلوروفيل في(38.14 SPAD) M2 ، تلتها(37.76 SPAD) M1 ، ثم M3 (33.62 SPAD). أُجري تحليل RAPD-PCR لتقييم التنوع الجيني، وتم الكشف عن 135 شريطًا جزيئيًا، منها 83 شريطًا مفقودًا و52 شريطًا فريدًا. سجلت M2 أعلى عدد من الأشرطة الفريدة (20)، تلتها(18) M3، ثم(14) M1. أما بالنسبة للأشرطة المفقودة، فقد كانت الأعلى في M2 (32)، ثم(29) M1 ، والأقل في M3 (22).توضح النتائج أن زيادة الملوحة تؤثر سلبًا على الإنبات والنمو والتنوع الوراثي، بينما تسبب الملوحة المتوسطة تنوعًا جينيًا أكبر من الملوحة العالية أو المنخفضة.

INTRODUCTION

Salinity is one of the most significant problems facing water resources [(1)], especially in central and southern Iraq, where it negatively affects the overall growth of plants and their ability to absorb nutrients[(2)]. Increased salinity in the soil leads to a reduction in the percentage of minerals that can be withdrawn by the plant [(3)]. Groundwater in Iraq is often about (97.5 %) saline [(4)]. Salinity is considered the primary issue for groundwater in Iraq due to its exposure to geological layers and the geochemical reactions that occur within them [(5)]. Additionally, there is the potential for contamination from agricultural fertilizers and municipal and industrial waste that can seep into groundwater through permeable layers, which limits water usage[(6)]. Increased salinity in irrigation water adversely affects plant growth, as it leads to osmotic stress. This stress makes it difficult for plants to absorb water, resulting in reduced vegetative mass and decreased plant height. Furthermore, high salinity can disrupt the physiological processes within plants, leading to symptoms such as leaf burn, chlorosis, and even plant death if the levels are severe enough [(7)]. The impact of salinity on crops varies widely among species, with some demonstrating greater tolerance than others. Understanding these variations is crucial for developing effective agricultural practices in saline environments [(8)] *Lepidium sativum* L., commonly known as garden cress, is an annual herbaceous

plant that belongs to the Brassicaceae family, which includes around 300 genera and 3000 species of plants. This plant typically grows to a height of about (30-40 cm). Its leaves are basal, petiolate, and feature a clear central vein. The upper leaves are sessile, and the leaf blade is thin with multiple white flowers. It reproduces by seeds [9] .This plant has medical importance because it contains vitamins and minerals such as calcium and iron. It helps improve digestive health, regulates bowel movements, acts as an anti-inflammatory, enhances cardiovascular health, and helps lower cholesterol levels in the blood [10]. The plant can be consumed raw, and the chemical compounds in garden cress have been noted for their resistance to various diseases. Some active compounds in garden cress oil are effective and can be used without side effects. Garden cress contains alkaloids, phenols, and other pharmaceutically and medically effective compounds, contributing to its high-quality natural efficacy[11] Random Amplified Polymorphic Deoxyribonucleic acid (DNA) markers are among the most commonly used molecular markers based on PCR, characterized by complete dominance, with the detection of genetic variations relying on the presence or absence of duplicated bands in individuals. Molecular markers are utilized to detect genetic variations within plant groups and to determine genetic relationships between varieties and species. Studies have shown that molecular markers are valuable indicators for describing and evaluating genetic diversity within species since they rely on DNA present in all cells of the organism [12]. These markers enable the detection of genetic variations among individuals, determining genetic relationships, distinguishing and diagnosing agricultural species and varieties, as well as early sex discrimination and studying the genetic stability of plants across multiple sites. They are also used for genetic fingerprinting and creating genetic maps for animals and plants, as well as determining the degree of similarity and differences among varieties within the same species. RAPD markers are the most common indicators among the multiple molecular indicators on PCR because they are characterized by complete dominance and the detection of genetic variations depends on the presence or absence of the multiple bands of individuals [13]. In addition to the ability of this indicator to detect more than one site on the gene when the primers are linked to more than one site [14], these indicators are also distinguished in determining the genetic fingerprint and determining the genetic maps of animals and plants, as well as determining the extent of similarity and difference between varieties belonging to the same species [15, 16]. This study aims to evaluate the effect of different concentrations of salts on the growth of *Lepidium sativum* L, phenotypic traits, molecular changes, and mutation rates. Through this research, we seek to understand how salinity impacts this plant and explore its genetic potential for salinity tolerance, which contributes to improving sustainable agricultural strategies in areas affected by increasing salinity.

MATERIALS AND METHODS

Experimental design

Water was obtained from wells located in Salah al-Din Governorate, Al-Alam District. These wells were at a depth of (24 m) and had different salinity ratios. Salinity was measured for all samples using a device Conductivity Multi-meter Hannah's type. The device is calibrated before using unit of measure mg/L shown in the Table (1). *Lepidium sativum* L. seeds were planted in the greenhouse affiliated with the College of Science / Department of Biology in pots and divided into 4 groups, each group containing 5 replicates. The first group (control) was planted with river water, while the remaining three groups were planted. The first group was planted with water from the first well with low hardness, the second group was treated with water with medium hardness, and the third group was treated with water with high hardness, respectively. After (45 days), the plants were

transferred to take measurements of phenotypic characteristics, extract DNA from the plant, and conduct RAPD-PCR reactions in the Molecular Biology Laboratory / College of Science / Tikrit University.

Table 1: The samples studied, the symbols used and the amount of hardness for each sample.

Treatment	Salinity concentration (mg/L)			
The symbols used	Control A	M1	M2	M3
The amount of Exposure	0.80	5.30	19.17	23.35

Sample collection for DNA extraction

The phenotypic characteristics were not measured nor was DNA extracted from these samples. Samples were collected from the plants after 45 days of cultivation under the influence of different concentrations of salinity (5.30, 19.17, 23.35 mg/L). (4-5) new apical meristematic leaves were taken and placed in special bags to transport the samples to the laboratory to isolate DNA from them.

DNA extraction: DNA was extracted from fresh leaves using the Cetyltrimethylammonium bromide (CTAB) method as described [17, 18]. DNA was purified according to the method of [19].

DNA concentration and purity measurement

DNA concentration and purity were determined using Nano drop, the sample was diluted to a concentration of (50 ng/µL) and then stored in the freezer until use.

Agarose gel electrophoresis

Materials, migration solution, gels and samples were prepared for electrophoresis according to the method of [20, 21].

RAPD_PCR parameters: RAPD_PCR reactions were performed for all *Lepidium sativum* L. plant samples using (9 primers) shown in Table (2) according to the method provided by [22].

Table 2: The primers used in the study.

no.	Primer	Sequence 5´→→→ 3´	no.	Primer	Sequence 5´→→→ 3´
1.	OP A-02	CAGGCCCTTC	6.	0P H-16	GTCCGATACA
2.	OP D-10	TGGACCGTGC	7.	0P B-20	GGGAGCATCC
3.	OP D-18	CCTTGACGCA	8.	OP B-12	CGGAACAGCA
4.	0P D-03	GGACACCGGT	9.	OP J-04	GAGAGCAACC
5.	OP D-12	TCTCAGCTGG			

Method of work

(Prepared by Premix Company by mixing RAPD-PCR the main reaction mixture was prepared all DNA plus (4 µL) of primer) with (2 µL) of Bioneer primer with (4 µL) of distilled water removed Master Mi the studied samples with (10 µL) ions and the final volume of the mixture becomes (20 µL) shown in Table (3).

After that, these tubes containing the mixture are placed in the thermal polymerization device and the program mentioned is applied and after the reaction is completed, the tubes are removed from the device and the samples are transferred to the electrophoresis device.

Table 3: Solutions used in the RAPD markers.

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

The PCR-RAPD markers

This is carried out according to the following steps: First, an initial denaturation of one cycle at (94 °C) for (4 min), then denaturation of 40 cycles at (93 °C) for (45 s), primer binding of 40 cycles at (36 °C) for (1 min), elongation at (72 °C) and 40 cycles for (1.5 min), and finally a final elongation of one cycle at (72 °C) for (10 min).

After completing the amplification in the PCR device, (4 µL) of the PCR-RAPD products are taken and transferred to a (1.5 %) agarose gel with DNA

Marker, after which the gel is imaged using ultraviolet (UV) rays [23]. The results of the multiplication operations of the primers used in the RAPD markers were taken and converted into tables, depending on the presence or absence of the DNA bands and comparing it between the different samples, where the presence of the bands is symbolized by the number (1) and the absence of the bands by the number (0) [19, 24].

RESULTS AND DISCUSSION

Results of phenotypic traits

The results showed that sample M1 was the first sample to grow, and after (24 hours), sample M2 grew, and after (72 hours), sample M3 grew. After the complete growth of *Lepidium sativum* L. plant, measurements of phenotypic traits were taken, which included (germination period, plant height, number of leaves, number of branches, chlorophyll content), as in Table (4).

Table 4: Morphological characteristics of *Lepidium sativum* L.

Sample	Plant Height(cm)	Number of Leaf	Germination time (hour)	Number of branches	Chlorophyll content (mg/g fresh weight)
Control sample (C)	26.15	18	16	7.3	37.51
Low salinity (M1)	30.67	23	18	6.7	37.76
Medium salinity (M2)	30.02	22	24	7.1	38.14
High salinity (M3)	16.28	10	72	5.5	33.62

Note: refers to the Soil Plant Analysis Development value, which is a measurement of chlorophyll content in leaves. The SPAD value is obtained using a SPAD meter.

The results of the morphological characteristics of the studied samples of *Lepidium sativum* L. showed that the highest plant height was in sample M1, which was (30.67 cm) compared to the control sample, followed by sample M2 with a height of (30.02 cm), and the lowest plant height was in sample M3, (16.28 cm). The highest number of leaves appeared in sample M1, which was (23 leaves) compared to the control sample, followed by sample M2, which was (22 leaves), and the lowest number of leaves was in sample M3, which was (10 leaves). Regarding the number of branches, the highest number was in sample M2, which was (7.1) compared to the control sample, followed by M1, which was (6.7), and the lowest number of branches was in sample M3, which was (5.5). As for the chlorophyll content, sample M2 excelled in the highest value, which reached (38.14 SPAD) compared to the control sample, followed by M1, which was (37.76 SPAD), and the lowest value was (33.62 SPAD) in sample M3.

Results of RAPD-PCR markers

The RAPD results showed that there were variations at the DNA level in most samples, and all samples were characterized by containing different mutations according to the effect of the substance with which the plant was treated. Most samples were characterized by containing unique bands and absent bands, as shown in Table (5). The results of the primers used in the study for the garden cress plant showed that the number of resulting sites was (93 sites), including (19) general sites in all treatments, and the different sites produced by these primers were (74 sites), and the total number of bands was (136 bands), while the results showed that the number of general bands was (76 bands), and the number of different bands was (135 bands), resulting from the sum of unique bands and absent bands, which were (52 bp) unique bands and (83 bp) absent bands. The results of Table (6) and Figurs (1 a–i) showed that the total number of different bands resulting in the studied samples was (135 bp), of which (83 bp) bands were absent and (52 bp) unique bands. The sample M2 treatment showed with

medium hardness water, the highest number of unique bands was (20 bp) unique bands, while sample M1 had the lowest number of unique bands, which was (14 bp) bands. As for the absent bands, the highest value appeared in the treated sample M2, where the number of absent bands was (32 bp) bands, while sample M3 showed the lowest number of absent bands, where their number was (22 bp) absent bands. The characteristics of the bands that were referred to for the effect of hardness on DNA and the appearance of absent bands in some ranges without others were due to the mutations produced in the designated sites on the DNA strand, and the occurrence of mutations in a specific site leads to the failure of the primer to bind due to the failure to recognize its complementary site as a result of the disappearance of some bases.

The results showed that the lower the concentration of salts in the irrigation water, the faster the germination rate, i.e. the proportion is inverse, which is consistent with what was mentioned by [24, 25], who showed that the factors determining the success of planting plants irrigated with saline irrigation water are the ability of seeds to germinate and form strong-growing seedlings that can withstand salt stress and the environmental conditions they face during their growth period. Seed germination is usually associated with water absorption, but water absorption from the saline medium at the beginning of the germination process is one of the main factors affecting the germination process, as it often leads to low water absorption, which leads to failure of germination and the formation of unhealthy seedlings. Salinity negatively affected many of the plant's phenotypic and vital characteristics, as excessive salinity caused a noticeable decline in plant height and number of leaves compared to the control sample. This occurred as a result of a decline in the rate of water absorption and an imbalance of nutrients. This is consistent with the study by [26], who showed that salinity has multiple effects on plant growth, and these effects include the direct effects of salts present in irrigation water or in the soil solution, which lead to hinder plant growth and reduce its productivity, and these effects include the osmotic effect and the toxic effect in addition to the ionic imbalance, and the damage caused by these effects on the plant can be through one or more of them in the plant growth and productivity and indirect effects by creating unsuitable conditions for plant growth as a result of the effect of salinity on the physical and chemical properties of the soil. The results of this study indicated that salinity greatly affects the growth and yield characteristics. The phenotypic indicators of plant growth, including plant height, number of leaves and number of branches, were inversely proportional to the salinity concentrations that the plant was exposed to, i.e. the lower the salinity concentration, the more the vegetative growth of the plant. This is similar to what [27] reached in his study that he conducted in Basra Governorate, as he noted that the presence of increased salinity levels in irrigation water caused a decrease in growth characteristics, and the highest number of leaves appeared in sample M1 with (23 leaves), followed by sample M2 and the lowest number of leaves in sample M3 with (10 leaves). Regarding the number of branches, the highest The number in sample M2 is (7.1) compared to the control sample, followed by M1 (6.7) and the lowest number of branches in sample M3 5.5. As for the chlorophyll content, salinity caused a decrease in the number of chloroplasts and a decrease in the amount of chlorophyll. There is no significant difference between the M2 and M1 samples in the highest value of chlorophyll content, while the lowest value is in the M3 sample exposed to the highest salinity concentration. These results are similar to what [27] reached, as they showed that the higher the level of salt concentration, the lower the chlorophyll content in the plant. As a result of the effect of salts on plants, interest has increased in studying the damage caused by salinity to the plant and how to resist salts in order to improve plant production in saline environments.

The results indicate that the *Lepidium sativum* L. genome is affected by hardness in all treatments and that the high efficiency of RAPD in detecting mutations with a small number of resulting bands is close to what was mentioned by [28, 29], unlike classical breeding methods that require two or three generations to track mutations [7]. The occurrence of mutations in the *Lepidium sativum* L. genome and the genetic difference between the treated samples in the control sample, this effect may be positive or negative on the phenotype and yield.

Table 5: Results of the primers used in RAPD reactions for samples.

C	Primer Number	Loci number	Monomorphic l ci	Polymorphic loci number	Bands number	Monomorphic bands number	Polymorphic band number	Unique bands	Absent bands
1	P1(OPA-05)	1	8	20	4	22	9	13	100
2	P2(OP B-16)	3	5	22	12	11	3	8	50
3	P3(OP B-9)	1	7	16	4	10	3	7	62
4	P4(OP C-07)	3	7	25	12	9	7	2	36
5	P5(OP C-13)	1	9	21	4	19	10	9	90
6	P6(OP D-17)	3	8	28	12	12	1	11	50
7	P7(OP D-01)	3	9	35	12	16	6	10	44
8	P8(OP E-14)	2	9	30	8	20	9	11	66
9	P9(OP E-11)	2	12	39	8	16	4	12	42
Total		19	74	136	76	135	52	83	60

Table 6: Distinctive bands the efficiency of the primers, and the discriminatory ability.

N	Primer Name	Molecular Wight	Distinctive bands in Ants samples					
			M1		**M2**		**M3**	
			unique	**Absent**	**unique**	**Absent**	**Unique**	**Absent**
1	**P1**	**300–2000bp**	5	3	4	3	4	3
2	**P2**	**400–1600bp**	0	2	3	2	2	0
3	**P3**	**200–1500**	1	2	4	1	2	0
4	**P4**	**200–1600**	1	1	1	5	0	1
5	**P5**	**300–1500**	2	3	4	3	3	3
6	**P6**	**100–1250**	3	0	6	1	5	0
7	**P7**	**300–1000**	3	2	4	2	3	2
8	**P8**	**250–1500**	3	3	3	3	5	3
9	**P9**	**200–1500**	4	2	3	0	5	2
			22	18	32	20	29	14
			43		52		40	
			135					

From a phenotypic point of view, salt stress has an inhibitory effect on the vegetative growth of the plant. The higher the salt concentration, the more the seeds are delayed in germination as a result of obstructing the absorption of water by the seeds due to the increased salt concentration in the soil medium. This increase in salt concentration results in dwarfing of the plant due to a defect in the physiological processes of the plant to resist the toxic effect of high salt ions. This leads to a decrease in the activity of the meristematic cells responsible for the elongation of the plant because the increase in salts affects the process of cell division and elongation, and the accumulation of toxic salt ions leads to spotting and burning of the edges of the leaves. Molecularly, free radicals resulting from the effect of salt stress interfere greatly with the work of nucleic acids and cause a defect in the process of cell divisions. These radicals destroy the plasma membranes of cells during the division process.

This defect affects the placement of nitrogenous bases in their correct place and causes damage to the correction system for the process of cell division and base construction. Through this, mutations occur in the genome of the garden cress plant *Lepidium sativum*. Correlation between phenotypic and molecular traits the phenotypic and molecular results showed that there is a correlation between them. The correlation appeared in treatment M2 between the number of unique bundles, the number of absent bundles, the number of branches, and the chlorophyll content. The higher the number of bundles, the higher these traits, while the results of treatment M1 showed that it contained the least number of unique bundles and the appearance of the fastest growth and the highest plant height and the highest number of leaves. The number of absent bundles was average between the highest value and the lowest value for the absent bundles. The results of treatment M3 showed that the unique bundles were average in number, while it showed the least number of absent bundles, the slowest growth, and the lowest plant height. Fewer leaves, fewer branches, and lower chlorophyll content.

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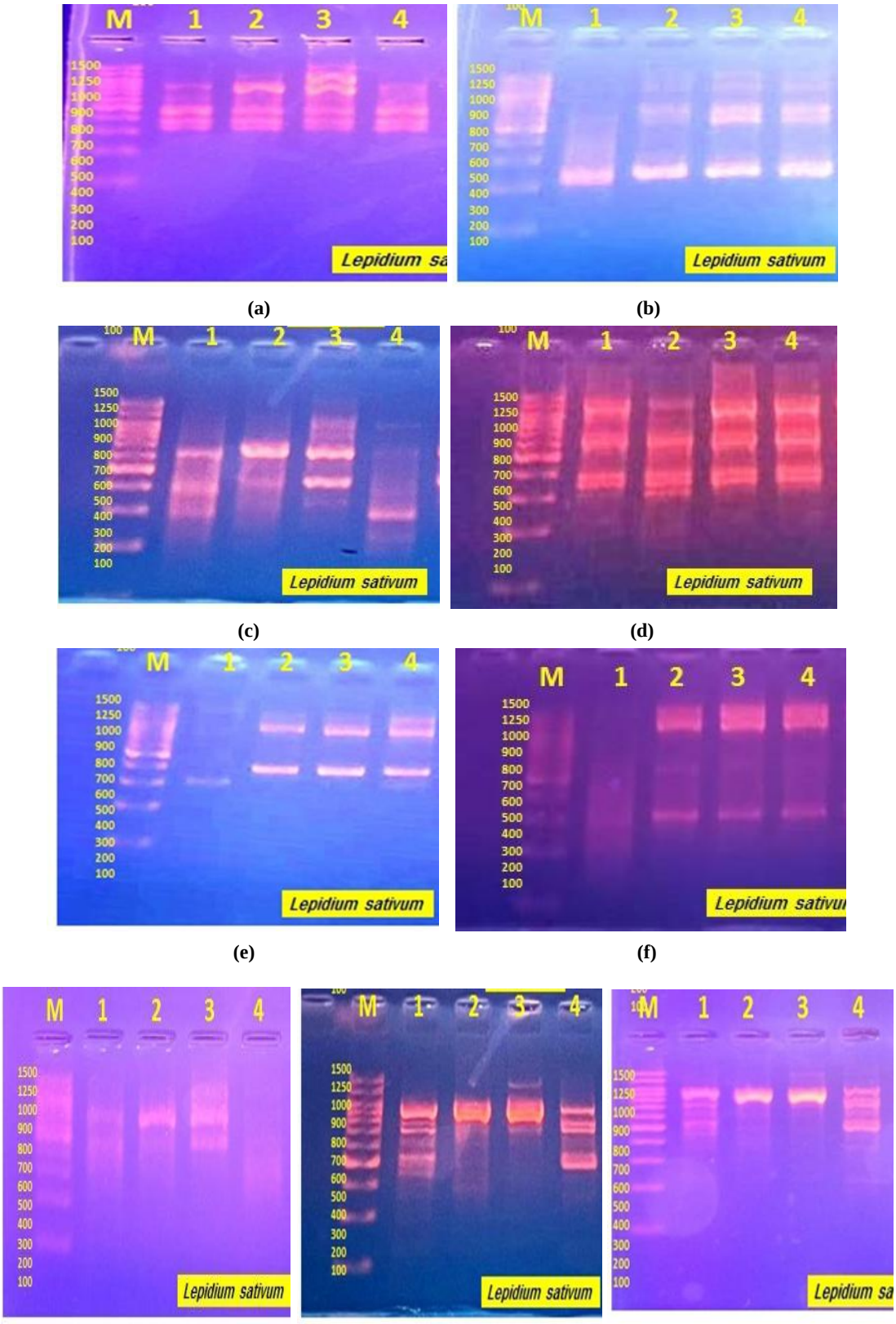

(a) **(b)**

(c) **(d)**

(e) **(f)**

(g) **(h)** **(i)**

Figur 1: The products of the primers of the four sample of *Lepidium sativum*: (a) P1, (b) P2, (c) P3, (d) P4, (e) P5, (f) P6, (g) P7, (h) P8, (i) P9.

CONCLUSIONS

Salt stress has an inhibitory effect on the vegetative growth of plants. Higher salt concentrations delay seed germination by hindering water absorption due to increased salinity in the soil. This results in stunted growth caused by physiological disruptions, including reduced activity of meristematic cells due to impaired cell division and elongation. The accumulation of toxic salt ions leads to leaf edge burning and spotting.

At the molecular level, salt-induced free radicals interfere with nucleic acid functions and disrupt cell division by damaging plasma membranes and altering the placement of nitrogenous bases. This damage affects the DNA repair system, leading to mutations in the genome of garden.

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