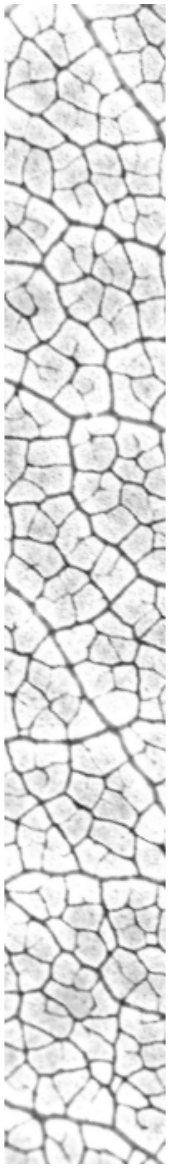

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Insertion red kidney beans in Iraqi food diversity program from production of cold tolerant lines by mutation technique

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Abstract
Two plant biotechnology experiments were conducted in institute of genetic engineering and biotechnology of high studies – university of Baghdad and college of sciences –university of Babylon, the other was in one of the opened greenhouse affiliated to ministry of agriculture to study new genotype of beans and its entering in food diversity program by the mutation of apex shoots of red kidney beans, the first experiment was included the irradiation of shoot apexes after its cut from shoots, the irradiation was by using ultraviolet radiation in three wavelengths (220, 320 and 400 nm) interaction with two exposure period (2 and 4 hours / day). The second experiment was cultured first and second generation seeds in five dates of planting in two seasons 2016/2017 and 2017/2018, the study traits of field experiment were: number of pods/plant, seed weight and plant seed yield. Randomized complete block design was used with three replications, the data was analyzed by using less significant difference test at 5% level, the results were: All mutant plants with different mutation treatments were given same results in second experiment, the CDKN1A gene was activated which inhibit kinase, it was cleared in D1, D2 and D5 treatments but it was silent in D3 and D4 treatment. D3 and D4 treatments was given significant growth and yield content: No. of pods per plant, seeds weight and plant seed yield in both the seasons, while D1, D2 and D5 treatments were not given yield, so new lines of red kidney beans are recommended in Iraqi food diversity program .

Keywords: irradiation, kidney bean, mutation, shoot apex, ultraviolet

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INTRODUCTION

Food diversity program is one of important program in the world which was using commercially in many establishments by entering new plant genotypes and species which are adaptive to the environments or treat to the adaptation, the new genotypes and species must have different taste, high production and have known cultivation conditions (Doronin *et al.* 2018), any plant must be entered in plant breeding programs and study of morphogenetic variations (Kumar *et al.* 2018), the study of red kidney beans is very important in this program because it do not culture in Iraq . Red kidney beans is one of important summer crop in many countries, it has high nutritional content from proteins, fibers, minerals and vitamins (Samuel and Ahiwe 2018), so that it is very important as new genotypes in crop rotation (Vakhnyi *et al.* 2018).

Many genes responsible for cellular metabolism need special conditions to activity (AL-Salihy and Jabbar 2017), and other conditions may be cause to silent genes, it seems plants can growth in range of conditions (AL-Salihy *et al.* 2018), this range different according to active genes and its continuous until plant complete life period, red kidney beans cannot growth in winter for growing in the summer (Balkaya and Odabaş 2007), but less of water irrigation and other problems in summer cause problem in the culture (Nejad *et al.* 2017).

Red beans has wide range of genes in genomic (Pandurangan *et al.* 2016), but all genes do not have activated , so it need some stress or shock may be activate of silent gene, some genes could not express because there are dominant genes which close genes, mutation technique is one of plant biotechnology use for the induction of genetic variations, it has also used as abnormal effective may be give continuous stimulation of genes or transformation of silent gene to active , there are two methods to the mutation: physical and chemical treatments, ultraviolet rays use in many studies to get changes in genetic materials, it has considered as environmental stress which effect on biological metabolism processes (Singh *et al.* 2014), it has been

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Table 1. The Heat degrees and Quantity of lighting of growth stages

Growth stages	Sites	Heat (ºC)	Light period (hour)
Culture apex in media	Incubator	10	10
Plantlets 2 cm	Incubator	14	11
Plantlets 4 cm	Incubator	14	11
Plantlets 6 cm	Incubator	16	12
Plantlets 15 cm	Incubator	18	12
Plantlets 20 cm	greenhouse	20±2	12
Plantlets 25 cm	greenhouse	24±2	14
Flowering 50%	greenhouse	26±2	14
Flowering 100%	greenhouse	28±2	

Table 2. PCR sample contents

Materials	Con. (µl)
Master mix	10
DNA	3
Forward primer	1
Reverse primer	1
Deionized distal water	5

caused changes in gene expression (Mckay *et al.* 2004), so this study was conducted to know role ultraviolet in stop one of dominant gene and ability to new lines of red kidney beans to grow in cold environments.

MATERIALS AND METHODS

There were two experiments in this study, the first experiment was conducted in institute of genetic engineering and biotechnology of higher studies – university of Baghdad and college of sciences – university of Babylon, second experiment was conducted in one of the opened greenhouse affiliated to Ministry of agriculture, the aim of study for entering new of summer kidney beans in food Iraqi people which can growth in winter season from activate gene expression under low heat stress by mutation technique , seeds were sterilized by ethanol 70% for 4 minutes and washed in sterilized distilled water and sterilized in sodium hypochlorite solution 5% with two drop of tween20 for 20 minutes, then it was washed sterilized distill water in three times, seeds were soaked in water over night for the germination and cultured in dishes, the dishes were incubated under 26 ± 2 ºC with 14 light: 10 dark (AL-Salihy and Jabbar 2017).

The mutation treatments were included three Wavelengths from ultraviolet rays (220, 320 and 400 nm) with two exposure periods (2 and 4 hours) , the exposure to mutation was after apex shoots cultured in media and incubated, U.V. light candles were used and connected with yellow candles in incubator, the exposure finished at Plantlets 15 cm.

Before exposure to mutation, the apex was cut after germination and cultured in vials, the media in vials was 4.9 g/L M.S salts, 100 mg/L of myo-inositol, 30 g/L sucrose, 7 g/L agar (M.S), plant hormones (5 mg/L N6-benzylamino purine + 1 mg/L 2,4-D) for growth apexes (Arias *et al.*,2010) , the program of incubater was in **Table 1**.

The field experiments was conducted in winter 2016-2017, 2017-2018, first and second generation seeds were cultured in pots 50 cm diameter included 2 seeds, then one plant in pot was let, randomized complete block design was used with three replications, the treatments were five dates of planting (D1: 30Dec., D2: 15Jan., D3: 30Jan., D4: 15Feb., D5: 01Mar.), the fertilization was included 30 kg N/ha with 70 kg P_2O_5/ ha (Wondimu and Tana 2017), study traits were: No. of pods/plant, seed weight (mg) and plant seeds yield (g) .

Detection of CDKN1A gene was included that fresh leaves collected from plants and it was used for RNA extraction, TRIzol (Invitrogen, U.S.A) was used for RNA extraction according to manufacturer's instructions, a total of 2 µg RNA was used for reverse transcription (RT) with the Transcript First-Strand cDNA synthesis super mix according to the manufacturer's instructions (Beijing TransGen Biotech Co., Ltd., China), PCR thermol program were contents for gene transcription in **Table 2**, the PCR program was remembered from (AL-Salihy *et al.* 2018), and many seconds as appropriate (60 s/kb) at 72°C; and 72°C for 10 min. The PCR product was analyzed by 1.5% agarose gel electrophoresis and one band was obtained.

Primers were CDKN1A F: AGGCACCATGTCCAATCC, CDKN1A R: AAGTCAAAGTTCCACCGTTCT. Statistical analysis of data was according to analysis of variance and the means tested by using L.S.D test under significant level 5% in SAS program (SAS 1992).

RESULTS AND DISCUSSION

After exposure plants to the mutation , there were changes in gene expression in some genes which became silent and others became active at end season, so it was necessary to study one of inhibitor metabolism gene for confirming the results , CDKN1A gene is one of metabolism gene which detected in kidney bean, this gene encoded a potent cyclin-dependent kinase inhibitor, the encoded protein binds and inhibited the activity of cyclin-cyclin-dependent kinase2 or -cyclin-dependent kinase4 complexes, and thus functions as a

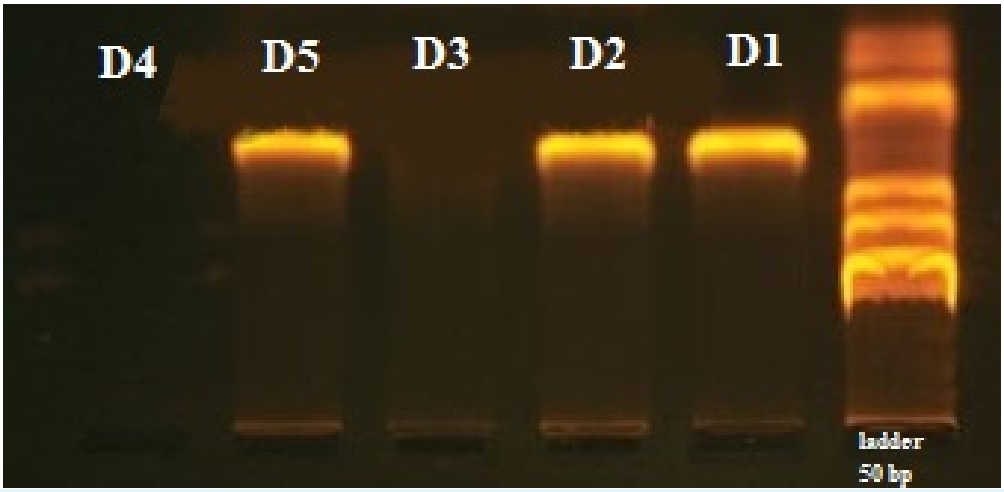

Fig. 1. Gel electrophoresis (1% agarose , 7 V/cm for 90 min) of *CDKN1A gene* in red kidney bean , first line : 50 bp DNA ladder, lines (1,2,4) positive results for *CDKN1A* gene

Table 3. Effect dates of planting in study traits

Sowing dates	2016-2017 season			2017-2018 season		
	No. of pods / plant	Seed weight (mg)	Plant seeds yield	No. of pods / plant	Seed weight (mg)	Plant seeds yield
D1	0	0	0	0	0	0
D2	0	0	0	0	0	0
D3	22.33	288	22.5	29.33	300.9	30.9
D4	19.5	234.2	16	24.67	279	24.1
D5	0	0	0	0	0	0
L.S.D	1.92	12.7	2.69	2.92	16.8	2.11

regulator of cell cycle progression at G1, so its gene expression had the ability to damage or missed cells, the appearance of the inhibitor gene is an evidence of plants went to the aging , so the gene detected in the early growth stages as evidence of the plant's inability to complete growth.

From the **'Fig. 1**' , the results refered to that the gene expressed in D1, D2 and D5 treatments from early growth stage as inhibitor to cells division caused as result with other factors dead plants as a reaction to the cold stress and mutation shock, while D3 and D4 treatments did not have gene expression of CDKN1A gene , this indicator refered to active of gene in dates of planting and the gene became silent in another as environmental adaptation under heat stress(Wang *et al.* 2017).

All plantlets which mutated in three wave length with two exposure periods were planted in five dates and the plants gave similar results in all dates of planting , so that study of different mutant seeds were unified . There were significant differences among dates of planting in all studies traits (2016-2017 and 2017-2018 seasons) appeared in **Table 3**, the results indicated inability (D1) seeds to complete the growth because its Weakness of portability to tolerance of low temperatures , while (D2) seeds could not complete growth because the high coldness at first stages, the (D5) seeds could growth and give stems , branches and leaves but it did not give yield Due to falling flowers from high temperatures at end season, it may be the mutation caused stopped in same genes responsible for tolerance of high temperatures, but the (D3, D4) seeds gave No. of pods / plant, Seed weight (mg) and seed yield. There were some genes responsible for tolerance of water and heat stress (Phiri 2015), non-specified cells may be had change in genetic material, if it exposed to high shock or mutation (Manaf *et al.* 2016), some inhibitor genes work under special conditions and this genes prevent or allow activate other genes responsible for tolerance of other conditions (khadeeva *et al.* 2009), so if inhibitor genes stopped, it might be allowed growth's plants in different environments, so that the exposure of genetic material to mutation led to non-stability and gave non-expectant results as plant death at very low temperature in (D1,D2) through the inhibition of some genes which were responsible for cold stress tolerance or high heat tolerance at flowering stage in (D5), the mutation caused stop some inhibitor genes which were dominant on other genes , this other genes became activity and allowed to plants growth under different environments in D3, D4 (Shaw and Chang 2005) in **Table 3**.

CONCLUSION

The results appeared new lines of red kidney beans which can growth in winter environments, mutation technique gave success in study throw the effect on activity of genes responsible for tolerance of low heat. Finally, this lines added as new winter crop enter into food diversity program in Iraq.

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