



RESPONSE OF F1 AND F2 GENERATIONS OF MUTANT BIG DARK SPECKLED KIDNEY BEANS TO COLD STRESS

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Abstract

Two experiments were conducted in institute of genetic engineering and biotechnology of higher studies – university of Baghdad and college of sciences – university of Babylon, the second experiment was conducted in one of the open greenhouse affiliated to ministry of agriculture, ultraviolet radiation was used in three wavelengths (220, 320 and 400 nm) interaction with two exposure periods (2 and 4 hours per day). In the second experiment, first generation seeds were planting in five sowing dates in two seasons 2016-2017 and 2017-2018, *atpA* gene was detected as genetic parameter, the study traits of morphological parameters were biological yield, number of pods/plant, number of seeds/pod, seed weight and plant seed yield, the results were in first season that D3 and D4 treatments were given yield traits in all seasons, it was biological yield with D5 treatment in all seasons, the *atpA* gene was appeared in D3 and D4 plants but it was silent in D5 plant at end season.

Key words: *atpA* gene, Generation, Kidney beans

Introduction

Adaptation and growth plants are the mix among genetics and ecology, their study has important as role in food security and human health. Lack of agriculture lands, natural resources, environmental degradation and climate changes, these problems lead to decrease of natural local food and increase of industrial food, to solve these problems, they need enter new species and breeds which have new taste and ability to the adaptation.

Speckled kidney beans is one of important economic summer crops in many countries, it has high nutritional content from proteins, fibers, minerals and vitamins (Samuel & Ahiwe, 2018), this breed do not culture in many middle east countries, while it has new taste to the people, the challenges of spread this breed has from lack of the knowledge of a breed and there are no institutions responsible for biodiversity and the ability to the adaptation has unknown, the lack of water irrigation and high temperatures in middle east sites make spread a breed very difficult, but if it can be tolerance to environmental stresses by the screening of genotypes (AL-Salihy & Jabbar, 2017), but other methods depend on changes in genetic material.

Genetic material depend on the gene appearance and gene expression, the genes have either active, silent or non-found (Saeed & Barozai, 2012), other studies indicate that silent genes have expressed under special conditions (AL-Salihy *et al.*, 2018), silent genes express by normal and abnormal external effects such as the mutations, mutation technique is one of biotechnology methods which cause genetic changes, for example, the UV light has given changes in chemical and growth traits (Manaf *et al.*, 2016), but any genetic change has cleared in non-specialized cells compared to specialized cells in the tissue, so they need study on activity of the genes by mutation technique to an adaptation, plant breeding and improvement programs use mutation technique for getting genetic variations in organism to adaptation of the stresses such as the cold environment, so this study was conducted by mutation of non-specialist cells (shoot apex) and its exposure to low heat environments for knowing its adaptation and study of genetic and morphological parameters in mutant big dark speckled kidney beans.

Materials and methods

Two experiments were conducted in institute of genetic engineering and biotechnology of higher studies – university of Baghdad and college of sciences –

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university of Babylon (first experiment), one of the greenhouses were affiliated to ministry of agriculture (second experiment) to study effect of mutation on shoot apexes and its growth under low heat environments to production of new winter big dark speckled kidney beans

First experiment

Seeds were sterilized by ethanol 70% for 4 minutes and they were washed in sterilized distilled water, then they were sterilized in sodium hypochlorite solution 5% with two drop of tween 20 for 20 minutes, then it was washed in sterilized distill water for three times, dry seeds were soaked in water over night for the germination, wet seeds were cultured in dishes, the dishes were incubated under $26 \pm 2^\circ\text{C}$ with 14 light: 10 dark (Arias *et al.*, 2010; Jabbar, 2018). After shoots were appeared, the apexes were cut and cultured in dishes, the dishes were included media from 4.9g.L^{-1} of M.S salts, 100mg.L^{-1} of myo-inositol, 30g.L^{-1} sucrose, 7g.L^{-1} agar and plant hormones 5mg.L^{-1} N6-benzylamino purine with 1mg.L^{-1} 2, 4-D

Table 1: The heat degrees and quantity of lighting in different growth stages.

Growth stages	Sites	Heat ($^\circ\text{C}$)	Light period (hours)
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	

for growth apexes (Murasgige & Skoog, 1962), after apexes grew, the plantlets were translocated at 4-6 cm length to small pots (5 cm diameter) which were included betemus and incubated under special environments, another environment incubates were explained in table 1.

Mutation treatments

Ultraviolet candles were included three Wavelengths (220, 320 and 400nm) with two exposure periods (2 and 4 hours), after apex shoots were cultured in media and incubated, U.V. light candles connected with yellow candles of incubator, the exposure finished at Plantlets 15cm.

Second experiment

This experiment was conducted in winter 2016-2017 and 2017-2018, the seeds were cultured in pots 50 cm dimeter, it was included 2 seeds per pot, then it was let one plant per pot, randomized complete block design was used with three replications, the treatments were five sowing dates (D1 : 30 Dec., D2 : 15 Jan., D3 : 30 Jan., D4 : 15 Feb., D5 : 01 Mar.), the fertilization was included 30kg N. ha^{-1} with $70\text{kg P}_2\text{O}_5\text{.ha}^{-1}$ (Wondimu & Tana, 2017), the study traits were : plant biological yield (g), No. of pods/plant, No. of seeds/pod, seed weight (mg)

Table 2: PCR sample contents.

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

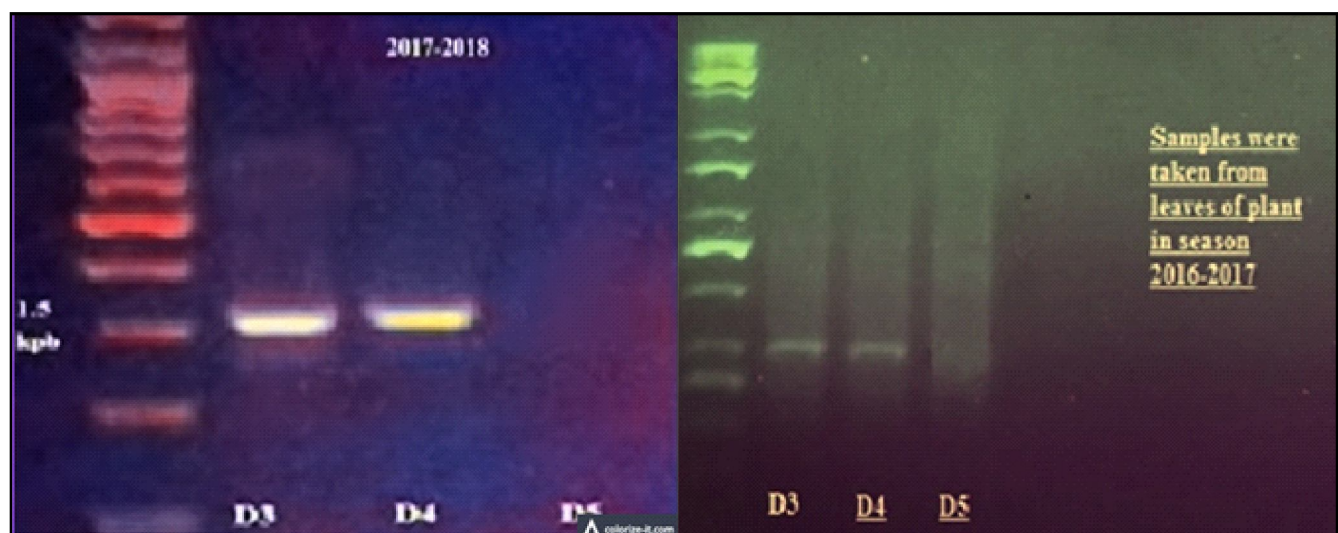


Fig. 1: Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of *atpA* gene in kidney bean in all seasons, first picture included first line : 100 bp DNA ladder, lines (2, 3) positive results for *atpA* gene, second picture included first line : 100 bp DNA ladder, lines (2, 3) positive results for *atpA* gene.

and plant seeds yield (g).

DNA extraction and PCR

Total genomic DNA was extracted according to the standard procedure of. (AL-Salihiy and Jabbar, 2017) with some modifications by Hamorabi kit (product by the institute of genetic engineering and biotechnology – university of Baghdad), type of PCR was thermocycler PCR, thermocycler PCR contents for gene transcription were explained in table 2, the PCR program was remembered from (D'Ovidio *et al.*, 1995), primers of ATP synthase CF1 alpha subunit gene were atpA F : GGAATTGTGGTCTTTGGTAGTG, atpA R : CCCCTCTTCCATCAATAGGTAC (Phiri, 2015).

Statistical analysis

The data was analysis according to analysis of variance and the means tested by using L.S.D test under significant level 5% in SAS program (SAS, 1990).

Results and Discussion

Genetic parameters

After exposure plants to mutation, the changes in gene expression of some genes became silent and others became active at end season, so it is necessary to study one of metabolism gene for confirming the results , the *atpA* gene is one of metabolism gene which detected in kidney bean, it has responsible for transcription of ATP synthase CF1 alpha subunit enzyme, this gene appeared in leaves of D3 and D4 treatments in end season (Fig. 1), it is index of the activity of metabolism genes in these treatments under theses environments, there were some genes responsible for tolerance of stresses (Phiri, 2015; AL-Salihiy *et al.*, 2018), the mutation of non-specified plant cells caused chemical changes connect with genetic changes (Manaf *et al.*, 2016), the mutation caused transformation some genes to silent and another silent genes expressed, the genes which expressed responsible for action of protease and peroxidase enzymes which

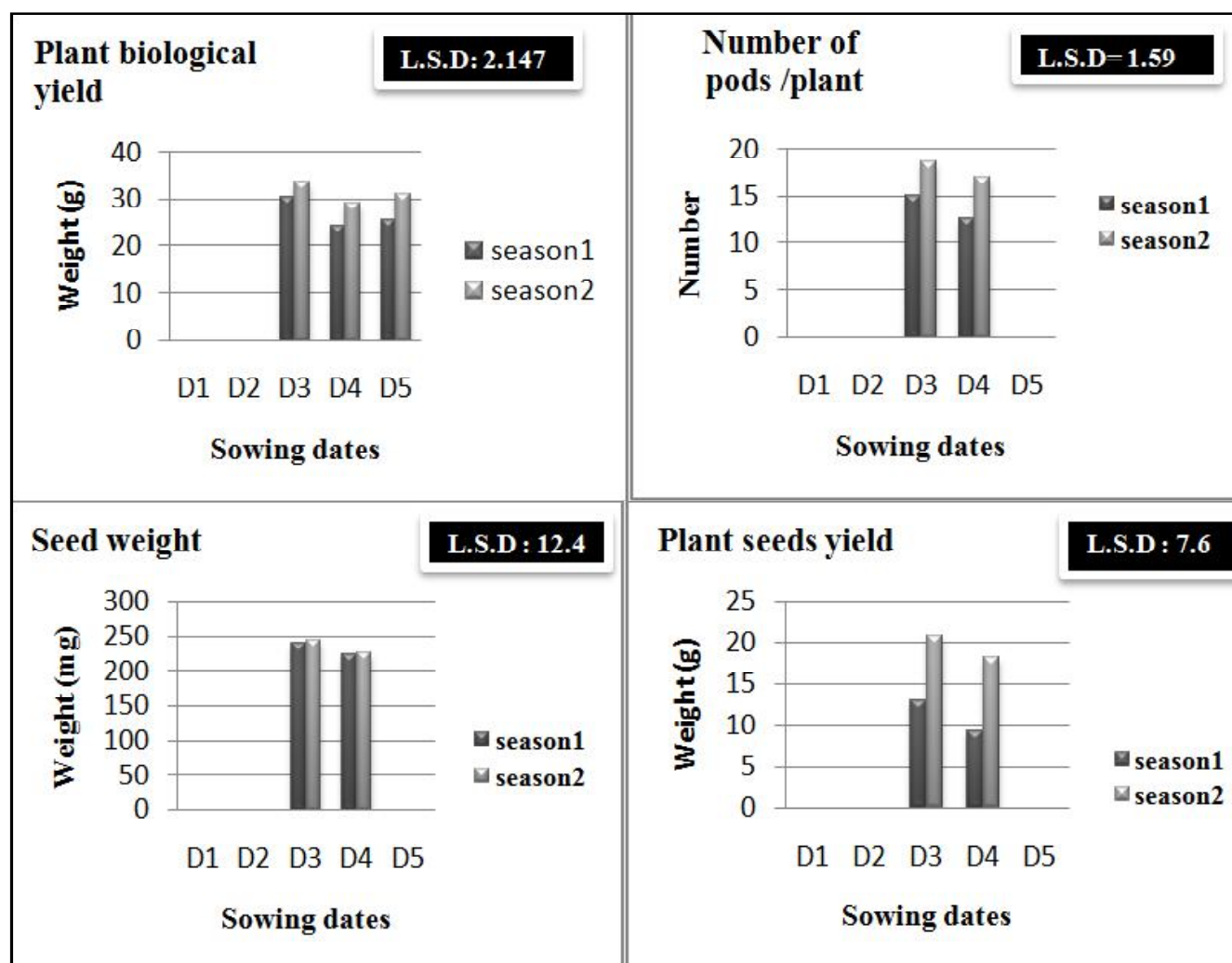


Fig. 2: Morphological parameters included plant biological yield, No. of pods/plant, seed weight and plant seeds yield in two seasons, first season : 2016-2017 and second season : 2017-2018, all parameters were significant under 5% level.

affected on growth (Peykarestan & Seify, 2012).

Morphological parameters

The mutation treatment (220nm × 2 hours) gave plants which could complete the season and gave yield. The first generation seeds of mutation treatments cultured in lines. There were significant differences among sowing dates in all studies traits appeared in Fig. 2, the results indicated inability D1 seeds to give plantlets from low temperatures after germination, D2 seeds could not give germination because the low temperatures found, the D5 seeds could growth and gave stems, branches and leaves but it did not give yield Due to fall the flowers from high temperatures at end season, but the D3 and D4 seeds gave biological and seeds yield, so it was very important to the focus in these treatment in the study, when plants could give growth and yield at D3 and D4 treatments, so they gave index on changes in ability to adaptation under different environments, in natural environments, plants could not growth in wide range of temperatures (Esmailzadeh & Amin-panah, 2015), so that exposure of genetic material to mutation led to non-stability and it may be gave non-expectant results as plant death at very low temperature in D1 and D2, this result agree with (Jabbar, 2019), the mutation activated silent genes or continuous in activity which affected on germination, growth and flowering (Shaw & Chang, 2005), these indicators refer to silent gene if they treated by any activation treatments, it become active and continue in activity as environmental adaptation under heat stress, these results found in D3 and D4 treatments (Jabbar, 2019), other genes became silent such as stop genes which are responsible for tolerance of high temperatures, this status found in D5 treatments.

Conclusion

The results appeared new breed of dark speckled kidney beans 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 breed will be added as new winter plant into food biodiversity in Iraq, so it need more studies about genetic stability.

Acknowledgements

I thank the institute of genetic engineering and biotechnology of higher studies – university of Baghdad and college of sciences – university of Babylon to allow complete this study in their laboratories.

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