Biochemical characteristics of red bean (*Phaseolus vulgaris* L.) genotypes as affected by seed pre-treatment with growth regulators

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Abstract

In order to investigate the effect of seed pretreatment with growth regulators on biochemical characteristics of red bean genotypes, a factorial experiment was carried out in a randomized complete block design with three replications. In this research, seed pretreatment with growth regulators namely salicylic acid (SA) and naphthalene acetic acid (NAA) were applied at four levels including P0: control (pretreated with distilled water); P1: pretreated with NAA hormone (0.5 mM/lit); P2: pretreated with SA hormone (0.7 mM/lit); P3: pretreated with combination of NAA hormones (0.5 and 0.7 mM/lit) and two genotypes of red beans (KS31169 and D81083). The ANOVA results showed that the simple effects and the interactive effects of genotypes × priming with growth regulators on all traits were significant except the relative chlorophyll content. The results of mean comparison for the interactive effect of genotypes and priming with growth regulators showed that application of SA increased the amount of chlorophyll a in D81083 genotype. The highest rate of chlorophyll b and total chlorophyll belonged to D81083 genotype with application of NAA. The rates of flavonoids and carotenoids decreased by using growth regulators, therefore the highest rates of these traits were observed in control × D81083 genotype. Seed priming with combined treatments (NAA+SA) led to the highest stability of cytoplasmic membrane in KS31169 genotype. In general, the results of the present study indicated that the use of growth regulators as seed pretreatment will increase the main pigments of photosynthesis and cytoplasmic membrane stability.

Keywords: chlorophyll; membrane stability; photosynthetic pigments; red beans; seed priming

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Introduction

The origin of bean (*Phaseolus vulgaris* L.) is central and South America and today it is planted in all tropical and temperate areas. By having 22-25% protein, bean is one of the most important sources of plant protein in many developing countries (Majnoon Hosseini, 2008). Practical seed priming which is common by the farmers consists soaking the seeds in water (often overnight) and surface drying and sowing the seeds in the same day (Clarke et al., 2001; Murungu et al., 2004). In fact,

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this simple treatment can lead to faster germination, better establishment, and increased grain yield in a number of plants in different environmental conditions (Rashid et al., 2006). The logical reason for the effectiveness of on-farm seed priming is reducing the required time for seed germination and it could allow the seedling to grow away from the bad conditions of the soil (Farooq et al., 2008). Priming stimulates the synthesis and activation of hydrolytic enzymes such as β and α-amylase (Varier et al., 2010). These enzymes provide the required energy for germination and emergence of seedling via the oxidation of seed storage nutrients.

Salicylic acid (SA) is a phenolic compound that exists in a large number of plants. This combination is considered today as a hormone-like substance and plays an important role in the growth and development of plants (Kang, 2003). Salicylic acid plays a central role in the regulation of various physiological processes during plant growth and development such as ion adsorption, photosynthesis, and germination, depending on the concentration used, species, growth period and environmental conditions. Seed priming with salicylic acid increased green index and percentage, leaf area, relative water content, photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, leaf proline content, and cell membrane stability, but reduced the number of days to flowering, the amount of CO_2 in stomata and soluble sugar content of the leaf (Senaranta et al., 2000). Naphthalene acetic acid (NAA) is an important synthetic auxin used in plants. Auxins have different effects on physiological processes of plants and the changes differ based on the concentration, chemical form of auxin, presence of other growth regulators, and even plant growth stage. Different effects of the application of NAA have been observed in acceleration of root development, control of flowering, prevention of fruit drop and increase of fruit formation, and fruit thinning in different plants (Prakash and Ganesan, 2000). Moreover, growth stimulators such as NAA can be useful by reducing the flower loss and enhancing the transition into the reservoir (Prakash et al., 2003). Given the role of growth regulators in metabolic and biochemical changes within plant cells and little research on the application of growth regulators as pretreatment for food legumes such as beans, the present research was conducted to investigate the effect of hormonal pretreatment on some biochemical changes of two red bean genotypes.

Material and Methods

This research was conducted in 2014-2015 in the National Bean Research Station of Khomein, Khomein, Iran. The station is located in the village of Khorramdasht, 8 km from Khomein-Qoorchi Bashi Road at longitude 49°57' E and latitude 33°2' and 1930 m above the sea level in the west of Khomein town. In the year before cultivation, wheat production has been carried out in the field. The soil (Table 1) was plowed in the autumn of previous year by mouldboard plow and in the spring two perpendicular discs were used. Before cultivation, 30 kg of phosphorus (based on 150 kg ha^{-1} of ammonium phosphate source) and 20 kg of Nitrogen from urea source (100 kg ha^{-1} as starter) were distributed in the land and mixed with soil by disc.

Table 1
The soil conditions and profile

Soil texture	Clay %	Silt %	Sand %	Br (P.P.M)	Cu (P.P.M)	Cu (P.P.M)	Zn (P.P.M)	Fe(P.P.M)	K (P.P.M)	P(P.P.M)	N %	OC%	T.N.V %	PH	E.C	SP %
SCL	22.4	31.6	46	1.26	1.04	6.72	4.16	2.98	313	23.4	0.05	0.37	24.1	8.1	0.5	29.6

A factorial experiment was carried out in a randomized complete block design with three replications. The research included two red bean genotypes (KS31169 and D81083) and seed pretreatment with growth regulators containing salicylic acid (SA) and naphthalene acetic acid (NAA) at four levels including P0: control (pretreated with distilled water); P1: pretreated with NAA hormone (0.5 mM lit^{-1}); P2: pretreated with SA hormone (0.7 mM lit^{-1}); P3: pretreated with combination of NAA hormones (0.5 and 0.7 mM lit^{-1}). For priming, after the preparation of different doses of salicylic acid and naphthalene acetic acid, red bean seeds were submerged under different treatments for six hours at the 4 ˚C. Then the seeds were dried at the room temperature and planting operations began in the field as direct cultivation. In this experiment, the distance between the rows was 50 cm and the distance between plants in each row was 5 cm. The length of each plot was 6 m and its width was 3 m and 6 rows were planted in each plot. During the vegetative growth stage, 50 kg ha^{-1} of urea fertilizer and 1 kg ha^{-1} of iron fertilizer were used with irrigation water before flowering. Weed control at different stages of growth and development of beans was done by manual weeding. Pest and diseases were controlled during the growing season in accordance with the technical recommendations.

Measuring chlorophyll and carotenoids

According to the method of Lichtenthaler et al. (1987), 0.1 g of leaf in each treatment was weighed accurately and was well-ground with 5 ml of acetone 80%. Then, the resulting extract was placed in centrifuge with 27000 rpm for 10 minutes and then 3 ml of the supernatant fluid was removed and its absorption was measured at three wavelengths of 663, 647, and 470 nm by a spectrophotometer (UV-VIS, Scinco). Afterwards, the rates of chlorophyll a, chlorophyll b and carotenoids were calculated using the following formula:

Ca = 12/25 (A 663) – 2/79 (A647)
Cb = 21/50 (A647) – 5/10 (A 663)
Chl.T=Chl.a + Chl.b
Car = (1000 (A470) – 1/82 (ca) – 85/02 (cb) 0/198

where, Ca, Cb, and Car are chlorophyll a, chlorophyll b, and carotenoids in mg g^{-1} FW, respectively.

Measuring the amount of flavonoids

Leaf disks obtained from the plant weighing 0.5 g were crushed in a porcelain mortar with ethanol (Ethanol 99% and glacial acetic acid (1)) and then centrifuged for 10 minutes at 4000 rpm. Then, samples were placed in hot water bath at 80 °C. The intensity of absorption was read at 300 nm wavelength and was calculated using the following formula and was expressed based on the

Table 2
Analysis of variance for the effects of hormonal treatments and red beans genotypes on their biochemical characteristics

S.O.V	Mean of squares (MS)								
	df	Chlorophyll	Chlorophyll b	Total chlorophyll (a+b)	Ratio of chlorophyll (a/b)	Relative content of chlorophyll	Carotenoids	Flavonoid	Cell membrane stability
Replication	2	0.42	0.45	0.28	0.007	0.07	670.7	22.22	666.94
Genotypes (G)	1	12.92**	2.14^{ns}	25.37**	0.03*	598.3**	514285.66**	319.74**	1114.52^{ns}
Hormonal priming (H)	3	13.78**	83.46**	115.38**	0.128**	1.78^{ns}	108515.7**	664.28**	43205.95**
G × H	3	29.33**	19.55**	79.46**	0.118**	0.22^{ns}	34612.74**	70.15*	6406.1**
Error	14	0.76	1.5	3.04	0.007	3.47	31126.2	8.45	759.25
Total CV%	23	13.57	10.21	9.46	14.84	4.53	13.36	6.13	12.18

percent of absorption p (absorbance %) (Krizik et al., 1998):

100 (V/700) fla = ABS (300nm)
where V is extract volume.

priming with salicylic acid. Mean comparison of the interactive effect of hormonal pretreatment and genotypes showed that the highest rate of chlorophyll a belonged to the D81083 genotype with salicylic pretreatment (Table 3). In general, hormonal priming in this experiment increased

Table 3
Mean comparisons of genotypes, hormonal treatments and their interaction in biochemical characteristics.

Treatment		Chlorophyll a	Chlorophyll b	Total chlorophyll (a+b)	Ratio of chlorophyll (a/b)	Relative content of chlorophyll	Carotenoids	Flavonoid
Genotypes (G)	KS31169	5.71b	11.70 b	17.41b	0.520 b	272.01 b	43.7 b	233.17 a
	D81083	7.17 a	12.29 a	19.47a	0.594 a	564.78 a	51.1 a	219.48 b
Hormonal priming (H)	distilled water (DW)	4.95 b	8.01 c	12.97 c	0.598 a	574.9 a	60.13 a	169.9 b
	NAA	5.87 b	16.82 a	22.70 a	0.347 b	256.0 c	45.43 b	207.1 b
	SA	7.53 a	12.64 b	21.16 a	0.688 a	453.4 b	49.40 b	176.9 b
	NAA+SA	6.41 b	10.52 b	16.93 b	0.595 a	389.3 b	34.67 c	351.3 a
G × H	KS31169 × DW	2.89 c	6.67 f	9.57 e	0.442 c	372.8 c	54.33 b	198.3 cd
	KS31169 × NAA	3.14 c	16.72 a	19.87 c	0.188 d	221.4 d	38.43 d	176.3 de
	KS31169 × SA	8.17 ab	10.73 cd	18.90 cd	0.763 a	270.1 d	47.07 c	167.4 de
	KS31169 × (NAA+SA)	8.64 ab	12.68 bc	21.32 bc	0.689 a	223.8 d	35.20 d	390.5 a
	D81083 × DW	7.02 b	9.36 de	16.38 d	0.754 a	776.9 a	65.93 a	141.5 e
	D81083 × NAA	8.61 ab	16.92 a	25.54 a	0.508 bc	290.7 cd	52.43 b	238.0 c
	D81083 × SA	8.89 a	14.55 b	23.87 ab	0.533 ab	636.6 b	51.73 bc	186.4 de
	D81083 × (NAA+SA)	4.91 c	8.36 ef	12.55 e	0.502 bc	554.8 b	34.13 d	312.0 b

Different letters within each column indicate significant difference at p≤0.05.

Results

Relative content of chlorophyll

The ANOVA results showed that only red bean genotypes were significantly different at 5% probability level (Table 2).

Chlorophyll a

The ANOVA results showed that the effect of genotypes and seeds priming with growth regulators and the interactive effect of genotypes × hormonal priming on the rate of chlorophyll a were significant at 1% probability level (Table 2). In this experiment, D81083 genotype had higher rate of chlorophyll a. Comparison of the means showed that at different hormonal priming levels the highest rate of chlorophyll a belonged to the

the rate of chlorophyll a and D18083 genotype has been influenced more than the other genotype. In addition, chlorophyll a increased by hormonal priming and D81083 genotype was unaffected by priming.

Chlorophyll b

The ANOVA results showed that the effect of seed priming with growth regulators and the interactive effect of genotypes × hormonal priming on the rate of chlorophyll b were significant at 1% probability level (Table 2). Comparison of the means showed that hormonal priming with just naphthalene acetic acid significantly increased chlorophyll b content (Table 3). Comparison of the interactive effects of genotypes and different levels of hormonal priming showed that both genotypes (KS31169

and D81083) had higher rate of chlorophyll b with application of naphthalene acetic acid than other hormonal treatments (Table 3).

Total chlorophyll (a + b)

The ANOVA results showed that the effect of red bean genotypes on total chlorophyll (a + b) was significant at 5% probability level and the effect of growth regulating hormonal priming and the interactive effect of genotypes × hormonal priming on the total chlorophyll (a +b) were significant at 1% probability level (Table 2). Mean comparison of the interactive effect of genotypes and hormonal pretreatments showed that the highest rate of total chlorophyll belonged to D81083×NAA (Table 3).

Ratio of chlorophyll (a/b)

The effect of red bean genotypes and the interactive effect of genotypes × hormonal priming on the ratio of chlorophyll (a/b) were significant at 1% probability level (Table 2). The results of mean comparison showed that the highest ratio of chlorophyll (a/b) belonged to D81083 genotype. Moreover, hormonal priming with naphthalene acetic acid had less effect on the ratio of chlorophyll. Mean comparison of the interactive effect of genotypes and hormonal pretreatments showed that the lowest rate of chlorophyll ratio belonged to the interactive effect of hormonal priming NAA × KS31169 genotype and the highest rate belonged to control × D81083 genotype which was not significantly different from the interactive effect of SA hormonal priming and compound treatment (SA+NAA) × genotype KS31169 (Table 3).

Carotenoids

The ANOVA results showed that the effect of different red bean genotype, seed priming with growth regulators, and the interactive effect of genotypes × hormonal priming on carotenoids were significant at 1% probability level (Table 2), so that the highest rate of carotenoids belonged to D81083 genotype and priming with distilled water (control) (Table 3).

Flavonoid

The effects of different red bean genotypes and seed priming with growth regulators on the rate of flavonoid were significant at 1% probability level, and the interactive effect of genotypes × hormonal priming on the rate of flavonoid was significant at 5% probability level (Table 2). The results of mean comparison showed that the highest rate of flavonoid belonged to D81083 genotype primed with distilled water. In fact, application of growth regulators decreased the flavonoid content (Table 3).

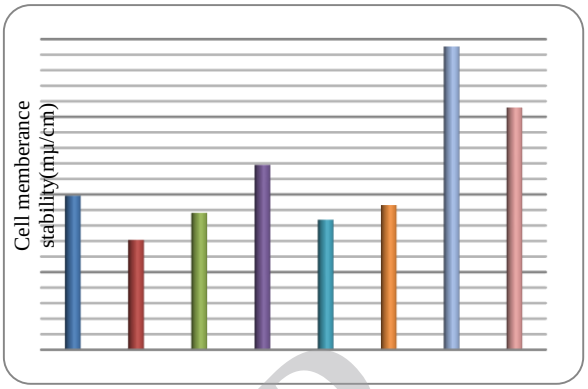

Fig. I. The changes of cell membrane stability at different levels of hormone treatments in two red bean genotypes ((salicylic acid (SA) and naphthalene acetic acid (NAA))

Cytoplasmic membrane stability index

The effect of seed priming with growth regulators and the interactive effect of genotypes × hormonal priming on cell membrane stability were significant at 1% probability level (Table 2). The results of mean comparison showed that seed priming with compound treatment (NAA+SA) increased cytoplasmic membrane stability and the interactive effect of genotypes × hormonal priming showed that the highest resistance of membrane belonged to KS31169 genotype and seed priming with compound treatment (NAA+SA) (Table 3).

Discussion

In the present study seed priming with growth regulators increased chlorophyll a, chlorophyll b, and total chlorophyll (a+b). Shivkumar et al. (2002) reported the increase of chlorophyll content due to the use of SA, and NAA

in pearl millet. Gharib (2006) reported that foliar spray of SA increased photosynthetic pigments (chlorophyll a and chlorophyll b) in basil and marjoram. As for the effect of SA on the content of photosynthetic pigments, Hayat et al. (2005) reported that in canola plants the increase in growth and photosynthetic pigments in response to SA pretreatment might be associated with the induction of antioxidant responses that protect the cells from oxidative damage resulting from stress. Singh and Usha (2003) also stated that soaking seeds in a solution of 1 mM salicylic acid would lead to the production of seedlings with higher chlorophyll content and rubisco activity compared with untreated seedlings.

Plants metabolic aspects which have been treated with salicylic acid showed changes based on the type of plant and the method of applying salicylic acid. For example, soaking wheat seeds in a solution of 0.1 M salicylic acid led to the production of plants which had higher amount of pigment and by increasing the concentration of salicylic acid, the amount of pigment decreased (Hayat, 2005). Turk et al. (2004) reported that spraying SA on bean plants increased chlorophylls a, b, and carotenoids under normal condition. Bagheri et al. (2013) reported the positive effects of application of naphthalene acetic acid on lengthening lifetime, increasing chlorophyll b content, decreasing sugar content due to the improvement of water and water absorption, controling chlorophyll degrading enzymes, and reducing breathing process in cut flowers of Alstroemeria.

Kang (2005) stated that the main role of carotenoids is to directly stop triplet chlorophylls and to prevent the production of single oxygen and ultimately to prevent oxidative damage. In fact, carotenoids do optical protection by lowering the excited state of chlorophyll. Hayat (2005) said that soaking mung bean seeds in an aqueous solution of salicylic acid led to decrease in the content of chlorophyll and carotenoid in the leaves.

According to Kim et al. (2009), total flavonoid content in plants of dandelion family in response to the application of growth regulators such as SA, cytokines, and gibberellic acid decreased the effect of these substances on the biosynthesis of secondary metabolism, significantly. In this study it was also observed that the composition of two plant growth regulators had the best effect on the flavonoid.

This present research showed seed priming with compound treatment (NAA+SA) increased cytoplasmic membrane stability. Studies showed that salicylic acid prevents damage to unsaturated fatty acids, reduces membrane permeability, protects cell membrane, and thus reduces electrolytes leakage (Borsanio et al., 2001).

Conclusion

In general, the results of this study showed that, application of seed priming technique using hormonal regulators and compounds enhanced the photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophyll (a+b). Because of the influence of growth regulators on the biosynthesis of secondary metabolites, the rate of flavonoids and carotenoids in red bean genotypes decreased, and the use of growth regulators led to the protection of cytoplasmic membrane.

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خصوصیات بیوشیمیایی ژنوتیپ های لوبیا قرمز (*Phaseolus vulgaris* L.) تحت تاثیر پیش تیمار بذر با تنظیم کننده های رشد

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چکیده فارسی

به منظور بررسی اثر پیش تیماربذر با تنظیم کننده های رشد (سالیسیک اسید و نفتالیک استیک اسید) بر صفات بیوشیمیایی ژنوتیپ های لوبیا قرمز، آزمایشی به صورت فاکتوریل بر پایه طرح بلوک های کامل تصادفی با سه تکرار اجرا شد. در این پژوهش، پیش تیمار بذر با تنظیم کننده‌های رشد شامل سالیسیلیک اسید (SA) و نفتالیک استیک اسید (NAA) در چهار سطح P۰: شاهد، پیش تیمار با آب مقطر؛P۱: پیش تیمار با هورمون NAA به میزان۰/۵میلی مولار در لیتر؛P۲: پیش تیمار با هورمون SA به میزان ۰/۷ میلی مولار در لیتر؛P۳: پیش تیمار با ترکیب دو هورمون NAA و SA به نسبت ۰/۵ و ۰/۷ میلی مولار و دو ژنوتیپ لوبیا قرمز KS31169 و D81083 مورد بررسی قرار گرفت. نتایج تجزیه واریانس صفات نشان داد که اثرات ساده و اثر متقابل ژنوتیپ × پرایمینگ تنظیم کننده‌های رشد بر تمامی صفات معنی دار بود، به جز محتوای کلروفیل نسبی که تنها تحت تاثیر ژنوتیپ های لوبیا قرار گرفت. نتایج مقایسه میانگین اثر متقابل ژنوتیپ × پرایمینگ تنظیم کننده های رشد نشان داد که کاربرد تنظیم کننده رشد SA در ژنوتیپ D81083 موجب افزایش میزان کلروفیل a شد. بیشترین میزان کلروفیل b و مجموع کلروفیل مربوط به ژنوتیپ D81083 با کاربرد NAA بود. با کاربرد تنظیم کننده های رشد میزان فلاونوئید و کارتنوئید کاهش یافت بطوری که بیشرین میزان این صفات درژنوتیپ D81083×شاهد مشاهده شد. کاربرد پرایمینگ بذر با تیمار ترکیبی (NAA+SA) بیشترین پایداری غشاء سیتوپلاسمی را در ژنوتیپ KS31169 موجب شد. بطور کلی، نتایج آزمایش حاضر گویای آن است که کاربرد تنظیم کننده های رشد به عنوان پیش تیمار بذر موجب افزایش رنگدانه های اصلی فتوسنتز و پایداری غشاء سیتوپلاسمی می‌شود.

کلمات کلیدی: کلروفیل، پایداری غشاء، رنگدانه های فتوسنتزی، لوبیا قرمز، پیش تیمار بذر