

Seed priming as a promising technique to improve growth, chlorophyll, photosynthesis and nutrient contents in cucumber seedlings

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Abstract

Seed priming is a technique to improve seed germination, seedlings growth, uniformity and yield. The present study was designed to, investigate the physiological mechanism of seed priming with GA₃ and KNO₃ on cucumber seedlings growth, chlorophyll, photosynthesis and nutrients uptake. The cucumber seeds were treated as; CK; control, T₁; GA₃ 100 ppm, T₂; GA₃ 200 ppm, T₃; KNO₃ 1%, T₄; KNO₃ 5%, before seed sowing. The results showed that seed priming with GA₃ and KNO₃ significantly increased the plant height, fresh and dry weight and strong seedling index. Moreover, chlorophyll a, chlorophyll b, chlorophyll a+b, carotenoid contents, net photosynthesis rate (P_n), stomatal conductance (G_s), transpiration rate (Tr) and intercellular CO₂ concentration in seed priming seedlings. In addition, seed priming significantly enhanced leaf macro and micro nutrient contents. Additionally, among various treatments GA₃ 200 ppm and KNO₃ 5% are found best. These results suggested that seed priming with GA₃ and KNO₃ synergistically promoted the chlorophyll contents, photosynthesis and nutrients uptake in cucumber seedlings, thus leading to improve plant growth.

Keywords: *Cucumis sativus* L; gibberellic acid; KNO₃; nutrients uptake; plant growth

Introduction

Seed priming is pre-sowing treatment that can improves seed germination performance and abiotic stress tolerance. The earlier studies showed that seed priming techniques are used for improvements of seed germination under normal and adverse environmental conditions (Jisha *et al.*, 2013) and useful physiological approaches that could adapt glycophyte species to saline conditions (Eskandari, 2013; Gholami *et al.*, 2015; Ruttanaruangboworn *et al.*, 2017). The positive effects of seed priming have been reported in many vegetable crops, such as tomato and pepper (Khan *et al.*, 2009; Muhammad *et al.*, 2010; Nakaune *et al.*, 2012). Seed priming also stimulates metabolic processes in pre-germinated seeds and makes active seed for radical protrusion and reduce physical resistance during imbibition of endosperm, repairs membranes and also improve developments of immature embryos (Khan *et al.*, 2009; Gamboa-Debuen *et al.*, 2010; Kubala *et al.*, 2015). Seed priming regulates various molecular, biochemical and physiological activities in seedlings (Yasutaka

et al., 2005; Nakaune *et al.*, 2012), including cell division, elongation and stress response proteins activation (Sivritepe *et al.*, 2003; Varier *et al.*, 2010; Mahajan *et al.*, 2011). Thus, it can be concluded that seed priming plays a key role in plant growth and developments.

Gibberellic acid (GA) is plant hormone that produces in root of plant and play essential role in plant growth and developments (Bai *et al.*, 2016). GA₃ play a significant role in regulation of plant growth and development at different environmental conditions (Muhammad and Eui Shik, 2007; Levent *et al.*, 2008). GA₃ is involved in improved seed germination, root growth, stem elongation and water and nutrient uptake. GA₃ pre sowing seed treatments are most effective for seedlings growth and nutrients uptake (Levent *et al.*, 2008; Bai *et al.*, 2016). Priming of GA₃ also stimulate seedlings sprouting, increase growth, activate enzymes (Muhammad and Eui Shik, 2007), that's are essential for carbohydrate metabolism, chlorophylls biosynthesis and other important enzymes, thus improve embryo developments, germination and seedlings growth, as previously reported by Varier *et al.* (2010). These findings are suggested that GA₃ is an important plant hormone that activates variety of developmental process during plan growth.

Nitrogen (N), potassium (K) and phosphorous (P) are major and essential nutrients for plant growth and development. The availability of these essential nutrients and uptakes are very important that effects growth. Plant possess a comprehensive transport system that is involved in uptake and transportation in nitrate, phosphate and potassium ion for soil though roots to shoots (Yi - Fang *et al.*, 2008). Moreover, these nutrients, nitrogen (KNO₃) plays an important function in balancing membrane potential and activating enzymes, and regulating osmotic pressure in cells (Chérel, 2004; de Jong *et al.*, 2014; O'Brien *et al.*, 2016), cell wall structure, cell elongation and cell division (Patade *et al.*, 2009). KNO₃ regulate uptake of nutrients across cell membranes and enhancing water uptake (Summart *et al.*, 2010). KNO₃ or CaCl₂ seed priming increased proteins, free amino acids and soluble sugars during germination under salt and water stress condition (Khan *et al.*, 2009). Acid phosphatase and phytase enzyme activities in the cotyledons, roots and shoots of lettuce under stress were increased by seed priming of KNO₃ (Nasri *et al.*, 2011).

Cucumber (*Cucumis sativus*. L) is important economic vegetable crop, it is originated from southern Asia, and widely cultivated in greenhouse during winter and summer seasons, as reported by Anwar *et al.* (2019). Because of high nutritional value, cucumber is very commonly cultivated and consumed all around the globe and mostly in China (FAO, 2017). Induced inhibition of seedlings growth caused by many environmental factors; light, temperature, and other abiotic stress, effects many physiological and biochemical process including reduction in photosynthesis, chlorophyll biosynthesis, nutrients uptake or imbalance in nutrients accumulation (Shah *et al.*, 2012; Yan *et al.*, 2013) and inhibition of biological carbon and nitrogen fixation (Meena *et al.*, 2016), thus leads in significant reduction in plant growth and yield of vegetable crops. Keeping in view the given facts, the current study was designed to evaluate the effect of GA₃ and KNO₃ seed priming at seedlings stage in cucumber. This study will provide deep understanding for protection and healthy vegetable nursery, and will be useful for protected vegetables production.

Materials and Methods

Plant material and experimental setup

The experiment was conducted at Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China. The cucumber (Cv. 'Zhongnong 26') seeds were treated with different levels of GA₃ and KNO₃; Control (CK) T₁ (GA₃ 100ppm), T₂ (GA₃ 200ppm), T₃ (KNO₃ 1%), T₄ (KNO₃ 5%). After 12 h (hours) treatment (the seeds were treated with various GA₃ and KNO₃ concentrations for 12 h) the seeds were dried at room temperature for 2 days. The seeds were grown in pots filled with soil mixture (Peat and vermiculite with 2:1, v/v). The experiment was conducted in control chamber having 14 h photoperiod (300

$\mu\text{mol. m}^{-2}\text{s}^{-1}$) at 25-28 °C during day time and 18-20 °C at night time for 10 h. After 7 days of seed germination the nutrients solutions were applied at same rate according to Yamazaki nutrients formula for cucumber seedlings (Table 1) with two days' interval.

Table 1. Yamazaki nutrition solutions and their concentration (mg/L) for cucumber seedlings

No	Name of compound	Concentration mg/L
A	Ca (NO ₃) ₂ ·4H ₂ O	35.4
	KNO ₃	40.4
B	NH ₄ H ₂ PO ₄	40.4
	MgSO ₄ ·7H ₂ O	24.6
C	Na ₂ Fe-EDTA	25.0
	H ₃ BO ₃	2.13
	MnSO ₄ ·4H ₂ O	2.86
	ZnSO ₄ ·7H ₂ O	0.22
	CuSO ₄ ·5H ₂ O	0.08
	(NH ₄) ₆ MO ₇ O ₂ ·4H ₂ O	0.02

Measurement of plant growth parameters

The same sizes of plants were selected after 15 days after seeds germination, to determined height and hypocotyl diameter using ruler and digital venire caliper respectively as described by Anwar *et al.* (2019). To determine fresh and dry weight, roots and shoots were separated and weighted as as described by Bai *et al.* (2016).

Chlorophyll contents measurement

To determine chlorophyll contents, fully expand fresh leave form each treatment (0.2 g) were homogenized in 95% ethanol for 24 h. The absorbance was read 665 nm, 649 nm and 470 nm, using spectrophotometer (Anwar *et al.*, 2018). The chlorophyll was calculated by using following formula:

$$\text{Chlorophyll a (mg/L)} = 12.21\text{OD}_{663} - 2.81\text{OD}_{646}$$

$$\text{Chlorophyll b (mg/L)} = 20.13\text{OD}_{646} - 5.03\text{OD}_{663}$$

$$\text{Carotenoid (mg/L)} = (1000\text{OD}_{470} - 3.27\text{Ca} - 104\text{Cb}) / 229$$

$$\text{Chlorophyll (a, b and Carotenoid) (mg/g. FW)} = C \cdot V \cdot n / W \quad (V=0.02 \text{ L, } n=1, W=0.2 \text{ g})$$

Measurement of photosynthesis

The net photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and intercellular CO₂ concentration of 3rd fully expanded leaf was determined by using portable photosynthesis system (LI-6400XT, LI-COR Lincoln, US) as described earlier (Anwar *et al.*, 2018).

Measurement of total nutrient contents

The total nutrient contents in plant leaves were determined by element analyser (Vario MAX CN Elemental Analyzer, Elementar, Hanau, Germany). The samples were 1st digested in HNO₃ by using microwave digestion system (Mars X press Microwave Digestion system, CEM, Matthews, NC, USA). Samples were then analysed for total nutrient concentrations with an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 5300 DV, Perkin Elmer, Waltham, USA), Jaldal Method were used for total N contents (Jiahui *et al.*, 2018).

Statistical analysis

There were four independent biological replications for each treatment, and the whole experiment was repeated four times. The data were statistically analyzed using an analysis of variance (ANOVA), and treatments were compared using the LSD test ($P=0.05$) using Statistix 8.1 software.

Results*Effect of seed priming on cucumber seedling growth*

Plant height, root length, hypocotyl diameter, fresh weight were significantly increased by GA₃ and KNO₃ seed priming as compare to control (CK) (Table 2). The results indicated that maximum plant height (13.33 cm) was noted T₄ treatments, followed significantly by T₂ and T₃ treatments, having plant height 12.33 cm. Minimum plant height (10.5 cm) was reported in control (CK). Moreover, T₂ had positively increase hypocotyl diameter as compared to control (CK), but the difference between T₁ and T₃ were reported non-significant. Fresh weight was also significantly increased in GA₃ and KNO₃ seed priming cucumber seedlings, as compared to CK. In this study T₂ was caused of notable increment in shoot, root and total fresh weight (Table 2). In addition, fresh weight 51.80% and 44% were increased in T₂ and T₄ respectively, as compared to CK.

Table 2. Effect of GA₃ and KNO₃ seed priming on plant height, root length, hypocotyl diameter and fresh weight

No	Plant height (cm)	Hypocotyl diameter (mm)	Shoot fresh weight (g)	Root fresh weight (g)	Total fresh weight (g)
CK	10.5±0.71 c	1.86±0.04 c	10.97±1.34 c	2.35±0.59 c	13.34±1.32 c
T ₁	11.67±0.57 b	2.14±0.06 b	13.25±0.44 b	2.51±0.20 bc	15.76±0.35 b
T ₂	12.33±0.60 ab	2.26±0.13 a	17.04±1.71 a	3.21±0.48 a	20.25±1.26 a
T ₃	12.33±0.60 ab	2.19±0.09 ab	15.85±0.89 a	3.06±0.27 ab	18.91±1.10 a
T ₄	13.33±1.15 a	2.17±0.05 ab	15.98±0.72 a	3.23±0.11 a	19.21±0.64 a

Data represent the mean values ± standard deviations. Different letters indicate significant differences at $P<0.05$. CK:

Control, T₁: GA₃ 100 ppm, T₂: GA₃ 200 ppm, T₃: KNO₃ 1%, T₄: KNO₃ 5%

Effect of seed priming on cucumber seedling dry weight

Seed priming with GA₃ and KNO₃ resulted in a significant increased dry weight of cucumber seedlings, as compared to CK, as presented in Table 3. The results indicated that among various treatments, shoot, root and total dry weight, (1.49 g, 0.24 g and 1.73 g respectively) were noted in T₄ treatment, whereas 1.55 g total dry weight was recorded in T₃. Dry weight was found lowest (1.23 g) in control (CK) treatment. Strong seedling index (SSI) was reported significantly higher in T₄ treatments (0.60), while lowest was noted in CK (0.43), but the difference between T₂ and T₃ were found non-significant. These findings are suggested that GA₃ and KNO₃ seed priming play important role in cucumber seedlings growth.

Table 3. The effect of GA₃ and KNO₃ seed priming on dry weight of shoot, root, total and root/shoot ratio

No.	Shoot dry weight (g)	Root dry weight (g)	Total dry weight (g)	SSI
CK	1.04±0.03 c	0.18±0.01 b	1.23±0.06 d	0.43±0.01 d
T ₁	1.20±0.07 b	0.18±0.02 b	1.39±0.07 bc	0.47±0.01 c
T ₂	1.27±0.01 b	0.19±0.03 b	1.45±0.01 c	0.53±0.02 b
T ₃	1.32±0.07 b	0.23±0.04 a	1.55±0.09 b	0.51±0.01 b
T ₄	1.49±0.012 a	0.24±0.03 a	1.73±0.01 a	0.60±0.02 a

Data represent the mean values ± standard deviations. Different letters indicate significant differences at $P<0.05$. CK:

Control, T₁: GA₃ 100 ppm, T₂: GA₃ 200 ppm, T₃: KNO₃ 1%, T₄: KNO₃ 5%

Effect of seed priming on cucumber seedling chlorophyll contents

Chlorophyll is the most important parameter that play an important role in photosynthetic capacity. In present study, the chlorophylls contents were significantly enhanced by GA₃ and KNO₃ seed priming, as presented in Figure 1. The results indicated that total chlorophyll contents (Chlorophyll a, b, a+b and carotenoid) were reported significantly higher in T₄ having 2.19, 0.89, 0.49 and 3.02 mg/g FW, respectively, followed by T₂ (2.09, 0.77, 0.47 and 2.86 mg/gFW) treatment in cucumber seedlings, but there is no significant difference between T₁, T₃, but significantly higher than CK, as presented in Figure 1. These findings are suggested the seed priming with GA₃ and KNO₃ significantly enhanced chlorophylls accumulation.

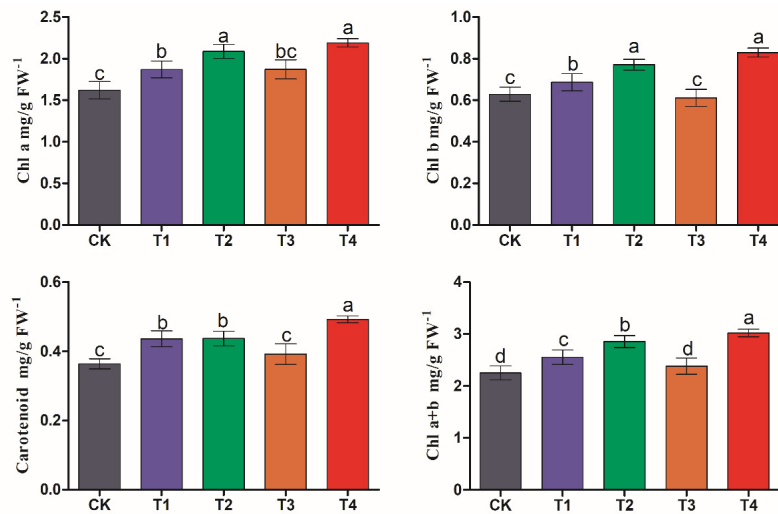


Figure 1. The effect of seed priming on chlorophyll contents in cucumber seedlings. Data represent the mean values \pm standard deviations. Different letters indicate significant differences at $P < 0.05$. CK: Control, T1: GA₃ 100ppm, T2: GA₃ 200ppm, T3: KNO₃ 1%, T4: KNO₃ 5%

Effect of seed priming on cucumber seedling photosynthesis

As shown in Figure 2, the GA₃ and KNO₃ seed priming significantly improved photosynthetic parameters including net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO₂ concentration (Ci) and transpiration rate (Tr) in cucumber seedlings. The results showed that Pn, Gs, Ci and Tr were increased by 55.47%, 88.23%, 51.04% and 171.82% in T₄ treatment and 45.80%, 52.94%, 56.98% and 98.89% were increased in T₃, when compared to CK. Whereas there is no significant difference between T₁, T₃ and CK. Similarly, Gs and Tr were found higher in T₁ and slightly decreased in T₄ and T₂, but remind statically higher than CK and T₃. It can be attributed that seed priming played important role in improvements of photosynthesis by increasing chlorophyll contents (Figure 1).

Effect of seed priming on total nutrients contents in cucumber leaves

The data presented in (Table 4), shows that GA₃ and KNO₃ seed priming significantly ($P = 0.05$) enhanced nutrients accumulation in cucumber leaves. The results showed that nutrients accumulations were significantly higher in seed priming of GA₃ and KNO₃ seedlings than control treatment (CK).

Macronutrient contents

The contents of total nitrogen (N) in the leaves of cucumber seedlings were significantly increased by 2.88% and 8.27% in T₂ and T₄ treatment respectively, as compare to CK treatment (Table 4). Moreover, T₁ and T₂ were also resulted in significant increment of N contents, when compared with CK. Seed priming had significantly enhanced P contents in leaves of cucumber seedlings. P contents in T₂ seedling leaves had increase by 6.97% and 13.95% increase were noted in T₄ treatment in comparison with untreated (CK) cucumber seedlings (Table 4). The K contents in cucumber leaves were significant improved by various seed priming treatments, as given in Table 4. The maximum increase K 37.9% and 35.2% reported contents were reported in T₂ and T₄ treatments respectively as compared CK. Mg and Ca contents were noted higher 49.7% and 40.7% in T₂ and 21.55% and 42.91% in T₁ respectively in leaves of cucumber seedlings, when compared with CK treatment. These findings are reflecting that seed priming played an important role in nutrient uptake and accumulation.

Table 4. Effect of seed priming on nutrients in leaves of cucumber seedling (mg/g DW⁻¹)

No.	CK	T ₁	T ₂	T ₃	T ₄
N	49.65±0.18 c	52.34±0.42 ab	51.08±1.78 bc	52.39±1.59 ab	53.76±1.13 a
K	6.16±1.04 b	6.16±1.07 b	8.50±0.55 a	8.94±0.01 a	8.33±1.14 a
P	0.86±0.02 b	0.92±0.00 ab	0.92±0.06 ab	0.84±0.11 b	0.98±0.17 a
Fe	0.12±0.02 c	0.13±0.01 c	0.17±0.02 b	0.21±0.00 a	0.18±0.01 b
Cu	0.005±0.00 b	0.005±0.00 b	0.007±0.00 a	0.007±0.00 a	0.007±0.00 a
Ca	5.15±0.65 b	5.64±1.28 ab	7.25±2.33 ab	7.36±0.67 a	5.35±0.33 b
Mg	1.67±0.20 b	1.70±0.21 b	2.50±0.41 a	2.65±0.11 a	2.03±0.18 ab
Ba	0.035±0.00 b	0.035±0.00 b	0.039±0.00 a	0.022±0.00 c	0.032±0.01 b
Na	0.65±0.09 c	0.69±0.10 c	1.06±0.04 a	0.97±0.02 b	1.35±0.99 a

Data represent the mean values ± standard deviations. Different letters indicate significant differences at $P < 0.05$. CK:

Control, T₁: GA₃ 100ppm, T₂: GA₃ 200ppm, T₃: KNO₃ 1%, T₄: KNO₃ 5%

Micronutrient contents

Seed priming also enhanced micronutrient contents in leaves of cucumber seedlings, are shown in Table 4. The results indicated that seed priming increased in Fe and Cu contents by 41.6% and 40% respectively in T₂ treatment, while 50%, and 40% respectively, were reported in T₄ treatment by comparing with CK treatment (Table 4). Similarly, Na contents in leaves of cucumber seedlings were also increased significantly in seed priming treatments. The Na contents were increased 63% and 107% in T₂ and T₄ respectively compared to CK. The B contents were increased in T₂, while noted lower in T₃ treatments, as shown in Table 4. Furthermore, it can be concluded that seed priming had positively enhanced seedlings growth by enhancing nutrients uptake. Taken together, seed priming treatment T₂ and T₄ resulted a significant increment in Chlorophyll contents, photosynthetic capacity and total nutrients contents, thus enhanced cucumber seedlings growth (Table 4).

Correlation analysis

The correlation analysis indicated that chlorophylls have positive correlation with plant growth indices, while showed negative correlation with root length and root/shoot ratio dry weight (Table 5). The photosynthesis (Pn, Gs, Ci and Tr) parameters reflect a positive correlation between cucumber seedlings growth, but chlorophyll, photosynthesis showed negative correlation between root/shoot ratio. In addition, photosynthesis parameters were showed maximum positive correlation with physiological parameters, than chlorophylls, but interactive correlation between chlorophyll and photosynthesis are found positive. These

results concluded that chlorophylls and photosynthesis produce changes in cucumber seedling growth, which was significantly enhanced in seed priming treatments.

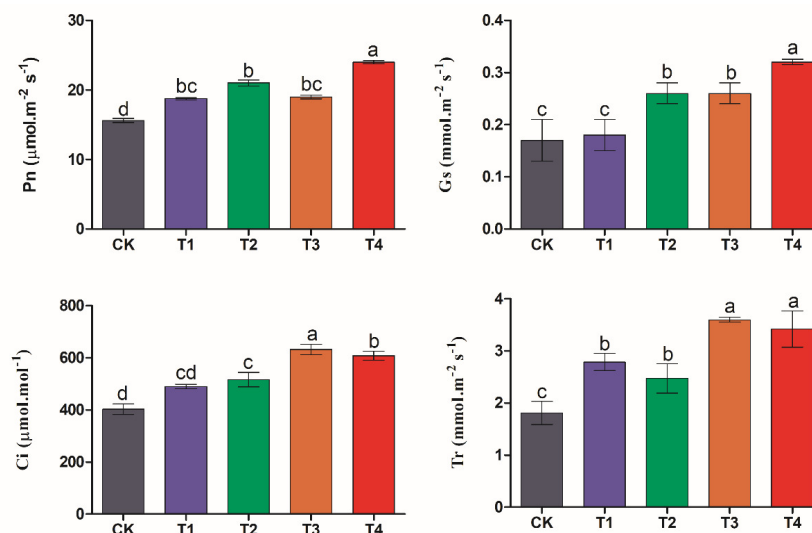


Figure 2. The effect of seed priming on photosynthesis in cucumber seedlings. Data represent the mean values \pm standard deviations. Different letters indicate significant differences at $P < 0.05$. CK: Control, T1: GA₃ 100 ppm, T2: GA₃ 200 ppm, T3: KNO₃ 1%, T4: KNO₃ 5%

Discussion

Seed priming techniques are recently used commercially to enhance seed vigor in term of germination and stress tolerance potential. In other words, it is also known as pre-germinative metabolism, because it activates metabolic process before seed germination, thus it showed a strong response to abiotic stress during germination. Seed priming extensively described, in term of plant ecological and physiological, cellular and molecular biology (Farooq *et al.*, 2007; Shah *et al.*, 2012; Sharma *et al.*, 2014). Recently, the increased quality of seed has become a key priority of agriculture market. Seed priming is also used to achieve rapid and uniform seed germination and seedling emergence to enhanced crop production performance. In the present study, we found that seed priming increase cucumber seedlings growth; height, hypocotyl diameter, shoot, fresh weight and dry weight as compared to CK treatment (Table 2, 3). The results were similar to those of earlier studies, which reported that seed priming enhanced watermelon and maize seedlings fresh weight, hypocotyl, when compared with unprimed seedlings (Demir and Mavi, 2004; Farooq *et al.*, 2007; Imran *et al.*, 2013). These findings are suggested that seed priming incredibly enhanced cucumber seedlings growth.

Chlorophylls are vital pigments that absorb a considerable amount of light energy and perform photosynthesis reactions in plant. Chlorophyll is very sensitive to various environmental stress, thus caused a significant reduction in chlorophyll contents and biosynthesis, thus effects plant growth and yield (Demir and Mavi, 2004; Khan *et al.*, 2009; Shah *et al.*, 2012). In the present study seed priming significantly increased chlorophyll contents in cucumber seedlings (Figure 1). The results are in agreement with previous studies, who reported that bio priming significantly enhanced chlorophyll contents in wheat leaves (Jiajin *et al.*, 2010; Rahimi, 2013; Siri *et al.*, 2013; Sharma *et al.*, 2014). Chlorophyll contents is an important parameter often used as an indicator for developments of chloroplast and photosynthetic capacity (Xia *et al.*, 2009; Bai *et al.*,

2016; Meena *et al.*, 2016), thus considered as a base for plant growth and developmental process. In present study, seed priming also enhanced N and Mg contents (Table 4), which is involved in chlorophyll biosynthesis. The Chlorophyll molecule contain Mg covalently link with four nitrogen (N) atoms (Chlorophyll a; $C_{55}H_{72}O_5N_4Mg$ and Chlorophyll b; $C_{55}H_{70}O_6N_4Mg$), so it might be the reason that seed priming enhanced nutrients uptake (Table 4) and resulted to enhanced chlorophyll contents in cucumber leaves. The correlation analysis, showed that chlorophyll have positive correlated with growth parameters and photosynthesis (Table 5). Taken together, these results suggested that seed priming enhanced chlorophyll contents thus leads to improve cucumber seedlings growth.

Table 5. Correlation analysis between plant different morphological parameters, chlorophyll and photosynthesis

Table 5 Pearson correlation analysis between plant different morphological parameters, chlorophyll and photosynthesis

	Plant height	Root length	Hypocotyl diameter	Shoot fresh weight	Root fresh weight	Total fresh weight	Shoot dry weight	Root dry weight	Total dry weight	Root/shoot ratio	Chlorophyll A	Chlorophyll B	Carotenoid	Chl. A+B	Pn	Gs	Ci	Tr
Plant height cm	100%																	
Root length cm	42%	100%																
Hypocotyl diameter mm	81%	69%	100%															
Shoot fresh weight g	87%	71%	93%	100%														
Root fresh weight g	90%	62%	81%	97%	100%													
Fresh weight g	88%	70%	92%	100%	98%	100%												
Shoot dry weight g	99%	36%	72%	80%	86%	81%	100%											
Root dry weight g	80%	42%	42%	59%	72%	61%	87%	100%										
Total dry weight g	97%	36%	67%	76%	83%	77%	100%	91%	100%									
Root/shoot ratio	-40%	9%	-58%	-30%	-14%	-28%	-34%	13%	-28%	100%								
Chlorophyll A mg/g FW	77%	-5%	63%	66%	68%	67%	72%	32%	66%	-62%	100%							
Chlorophyll B mg/g FW	74%	-22%	43%	52%	61%	53%	72%	41%	69%	-48%	96%	100%						
Carotenoid mg/g FW	67%	-21%	51%	53%	55%	53%	62%	21%	56%	-64%	99%	96%	100%					
Chlorophyll A+B	76%	-9%	59%	64%	67%	64%	71%	32%	65%	-59%	100%	97%	99%	100%				
Pn	97%	55%	89%	88%	86%	88%	95%	75%	93%	-48%	67%	59%	56%	65%	100%			
Gs	95%	44%	69%	86%	95%	88%	95%	86%	95%	-13%	71%	71%	60%	70%	88%	100%		
Ci	87%	68%	72%	78%	80%	79%	89%	90%	90%	-13%	36%	33%	22%	34%	91%	85%	100%	
Tr	89%	20%	50%	57%	67%	58%	95%	91%	97%	-25%	55%	64%	48%	55%	84%	85%	85%	100%

The mean data representing different plant growth effected by seed priming. Deep red color (100%) are representing strong positive correlation and deep blue color are representing negative correlation. The negative correlation are shows non-significant correlation with other parameters.

Photosynthesis is the base of plant growth and developmental process, while its capacity is often mainly ascribed to stomatal and non-stomatal limitations, and caused of reduction in Pn capacity, as well as Gs and Ci (Shu *et al.*, 2016). In this study, Photosynthetic activities (photosynthesis parameters including intercellular CO_2 concentration (Ci), photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr)) were significantly improved by seed priming in cucumber seedlings, as compared to CK (Figure 2). Chlorophylls accumulation are important parameter frequently used as an indicator of photosynthetic capacity, in present study we reported that seed priming enhanced chlorophylls accumulation (Figure 1), resulted a significant increment in photosynthetic capacity (Figure 2). The previous study reported a strong correlation between leaf N and chlorophylls, and concluded that photosynthesis capacity is frequently attributed to chlorophyll and N accumulation in leaf (Castro *et al.*, 2014), which is a key molecule for photosynthesis. In the present study, we also reported that seed priming enhanced N and Mg accumulation in cucumber leaf (Table 4). The previous study reported, that N and Mg are key molecule of chlorophyll

biosynthesis, thus it might be reason that seed priming enhance leaf nutrients accumulation (N and Mg) (Kutík *et al.*, 1995; Lawlor, 2002), and ultimate enhanced chlorophylls accumulation (Figure 1), and photosynthetic capacity (Figure 2) to improve cucumber seedlings growth (Table 2). The results were similar to those of earlier studies, which reported that seed priming with Salicylic acid and PEG enhanced photosynthesis in rice seedlings (Li and Zhang, 2010; Shaheen *et al.*, 2016), and Zhang *et al.* (2012), who reported that photosynthesis capacity in cucumber plant are increased by seed priming and also increased photochemical efficiency of PS II.

Sustaining ion homeostasis by nutrients uptake and compartmentalization is not only fundamental for plant growth (Ghars *et al.*, 2008), but also essential for almost all metabolic and cellular functions, such as energy metabolism, primary and secondary metabolism, regulation of genes, hormonal regulation, cell protection, reproduction, other signal transduction (Hansch and Mendel, 2009; Meena *et al.*, 2016). In this study, GA₃ and KNO₃ seed priming resulted a significant increment in minerals nutrition concentration (N, P, K, Mg, Ca, Cu, Fe, Na and Ba) in leaves of cucumber seedlings (Table 4). The results were similar to those of earlier studies, which reported that seed priming enhanced leaf nutrients accumulation and significantly enhance seedlings growth in mungbean (Shah *et al.*, 2012). Seed priming enhanced significantly nutrients uptake and balancing of membrane potential and regulating of osmotic pressure cells (Chérel, 2004), thus it might be reasoning that seed priming regulate nutrients uptake, chlorophylls accumulation and photosynthesis, leads significant increment in cucumber seedlings growth (Tables 1, 2).

Conclusions

Taken together, we presented physiological and biochemical evidences of seed priming regulate plant growth. As expected, cucumber seedlings growth was found significantly increased GA₃ and KNO₃ seed priming treatments, as compared to control. The KNO₃ and GA₃ seed priming had improved cucumber seedlings growth, fresh and dry weight, total chlorophyll contents, photosynthetic activity and leaves nutrients contents. Moreover, the personal correlation analysis concluded that chlorophyll and photosynthesis have positive correlation with plant height, hypocotyl dimeter, fresh and dry weight, and suggested that, seed priming regulates nutrient uptake, which involved in chlorophyll biosynthesis and photosynthesis, thus regulate cucumber growth. Among the treatments T₂ (GA₃ 200ppm) and T₄ (KNO₃ 5%) seed priming was found superior and recommended for improved seedlings growth of cucumber. The finding could improve our understanding of seed priming and would be useful for healthy nursery rising and protected vegetable production.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Anwar A, Bai L, Miao L, Liu Y, Li S, Yu X, Li Y (2018). 24-epibrassinolide ameliorates endogenous hormone levels to enhance low-temperature stress tolerance in cucumber seedlings. *International Journal of Molecular Sciences* 19(9):2497.
- Anwar A, Li Y, He C, Yu X (2019). 24-epibrassinolide promotes NO_3^- and NH_4^+ ion flux rate and NRT1 gene expression in cucumber under suboptimal root zone temperature. *BMC Plant Biology* 19(1):225.
- Anwar A, Yan Y, Liu Y, Li Y, Yu X (2018). 5-aminolevulinic acid improves nutrient uptake and endogenous hormone accumulation, enhancing low-temperature stress tolerance in cucumbers. *International Journal of Molecular Sciences* 19:3379.
- Bai L, Deng H, Zhang X, Yu X, Li Y (2016). Gibberellin is involved in inhibition of cucumber growth and nitrogen uptake at suboptimal root-zone temperatures. *Plos One* 11:e0156188.
- Castro FAD, Camprotrini E, Netto AT, Gomes MDMDA, Ferraz TM, Glenn DM (2014). Portable chlorophyll meter (PCM-502) values are related to total chlorophyll concentration and photosynthetic capacity in papaya (*Carica papaya* L.). *Theoretical and Experimental Plant Physiology* 26:201-210.
- Chérel I (2004). Regulation of K^+ channel activities in plants: from physiological to molecular aspects. *Journal of Experimental Botany* 55:337-351.
- De Jong F, Thodey K, Lejay LV, Bevan MW (2014). Glucose elevates nitrate transporter 2.1 protein levels and nitrate transport activity independently of its hexokinase I-mediated stimulation of nitrate transporter 2.1 expression. *Plant Physiology* 164:308-320.
- Demir I, Mavi K (2004). The effect of priming on seedling emergence of differentially matured watermelon Matsum and Nakai) seeds. *Scientia Horticulturae* 102:467-473.
- Eskandari H (2013). Effects of priming technique on seed germination properties, emergence and field performance of crops: a review. *International Journal of Agronomy and Plant Production* 4(3):454-458.
- Farooq DM, Basra SMA, Khan MB (2007). Seed priming improves growth of nursery seedlings and yield of transplanted rice. *Archives of Agronomy and Soil Science* 53:315-326.
- Gamboa-Debuen A, Cruz-Ortega R, Martínez-Barajas E, Sánchez-Coronado ME, Orozco-Segovia A (2010). Natural priming as an important metabolic event in the life history of *Wigandia urens* (Hydrophyllaceae) seeds. *Physiologia Plantarum* 128:520-530.
- Ghars MA, Parre E, Debez A, Bordenave M, Richard L, Lepot L, Bouchereau A, ... Abdelly C (2008). Comparative salt tolerance analysis between *Arabidopsis thaliana* and *Thellungiella halophila*, with special emphasis on K^+ / Na^+ selectivity and proline accumulation. *Journal of Plant Physiology* 165:588-599.
- Gholami M, Mokhtarian F, Baninasab B (2015). Seed haloprimer improves the germination performance of black seed (*Nigella sativa*) under salinity stress conditions. *Journal of Crop Science and Biotechnology* 18:21-26.
- Hansch R, Mendel RR (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology* 12:259-266.
- Imran M, Mahmood A, Römhild V, Neumann G (2013). Nutrient seed priming improves seedling development of maize exposed to low root zone temperatures during early growth. *European Journal of Agronomy* 49:141-148.
- Jiahui Z, Ning Z, Congcong L, Hao Y, Meiling L, Guirui Y, ... Nianpeng H (2018). C:N:P stoichiometry in China's forests: from organs to ecosystems. *Functional Ecology* 32:50-60.
- Jiajin Z, Weixiang W, Yun L, Wu X, Wang X (2010). Osmoprimer-regulated changes of plasma membrane composition and function were inhibited by phenylarsine oxide in soybean seeds. *Journal of Integrative Plant Biology* 51:858-867.
- Jisha KC, Vijayakumari K, Puthur JT (2013). Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum* 35:1381-1396.
- Khan HA, Ayub CM, Pervez MA, Bilal RM, Shahid MA, Ziaf K (2009). Effect of seed priming with NaCl on salinity tolerance of hot pepper (*Capsicum annuum* L.) at seedling stage. *Soil and Environment* 28:81-87.
- Kubala S, Garnczarska M, Wojtyła Ł, Clippe A, Kosmala A, Żmieńko A, ... Quinet M (2015). Deciphering priming-induced improvement of rapeseed (*Brassica napus* L.) germination through an integrated transcriptomic and proteomic approach. *Plant Science* 231:94-113.

- Kutik J, Nátr L, Demmersderks HH, Lawlor DW (1995). Chloroplast ultrastructure of sugar beet (*Beta vulgaris* L.) cultivated in normal and elevated CO₂ concentrations with two contrasted nitrogen supplies. *Journal of Experimental Botany* 46:1797-1802.
- Lawlor D (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany* 53(370):773-787.
- Li X, Zhang L (2012). SA and PEG-induced priming for water stress tolerance in rice seedling. In: *Information technology and agricultural engineering*. Springer, Berlin, Heidelberg pp 881-887.
- Mahajan G, Sarlach RS, Japinder S, Gill MS (2011). Seed Priming effects on germination, growth and yield of dry direct-seeded rice. *Journal of Crop Improvement* 25:409-417.
- Meena SK, Rakshit A, Meena VS (2016). Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (*Triticum aestivum* L.) under greenhouse conditions. *Biocatalysis and Agricultural Biotechnology* 6:68-75.
- Farooq M, Basra SM, Wahid A, Ahmad N (2010). Changes in nutrient-homeostasis and reserves metabolism during rice seed priming: consequences for seedling emergence and growth. *Agricultural Sciences in China* 9(2):191-198.
- Jamil M, Rha ES (2007). Gibberellic acid (GA₃) enhance seed water uptake, germination and early seedling growth in sugar beet under salt stress. *Pakistan Journal of Biological Sciences* 10(4):654-658.
- Nakaune M, Hanada A, Yin YG, Matsukura C, Yamaguchi S, Ezura H (2012). Molecular and physiological dissection of enhanced seed germination using short-term low-concentration salt seed priming in tomato. *Plant Physiology and Biochemistry* 52:28-37.
- Nasri N, Kaddour R, Mahmoudi H, Olfa B, Karray-Bouraoui N, Lachaal M (2011). The effect of osmopriming on germination, seedling growth and phosphatase activities of lettuce under saline condition. *African Journal of Biotechnology* 10(65):14366-14372.
- O'brien J, Vega A, Bouguyon E, Krouk G, Gojon A, Coruzzi G, Gutiérrez R (2016). Nitrate transport, sensing, and responses in plants. *Molecular Plant* 9:837-856.
- Patade VY, Bhargava S, Suprasanna P (2009). Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. *Agriculture, Ecosystems and Environment* 134:24-28.
- Yacoubi R, Job C, Belghazi M, Chaibi W, Job D (2011). Toward characterizing seed vigor in alfalfa through proteomic analysis of germination and priming. *Journal of Proteome Research* 10(9):3891-3903.
- Rahimi A (2013). Seed priming improves the germination performance of cumin (*Cuminum cyminum* L.) under temperature and water stress. *Industrial Crops and Products* 42:454-460.
- Ruttanaruangboworn A, Chanprasert W, Tobunluepop P, Onwimol D (2017). Effect of seed priming with different concentrations of potassium nitrate on the pattern of seed imbibition and germination of rice (*Oryza sativa* L.). *Journal of Integrative Agriculture* 16:605-613.
- Shah H, Jalwat T, Arif M, Miraj G (2012). Seed priming improves early seedling growth and nutrient uptake in mungbean. *Journal of Plant Nutrition* 35:805-816.
- Shaheen HL, Iqbal M, Azeem M, Shahbaz M, Shehzadi M (2016). K-priming positively modulates growth and nutrient status of salt-stressed cotton (*Gossypium hirsutum*) seedlings. *Archives of Agronomy & Soil Science* 62:759-768.
- Sharma AD, Rathore SVS, Srinivasan K, Tyagi RK (2014). Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). *Scientia Horticulturae* 165:75-81.
- Shu S, Tang Y, Yuan Y, Sun J, Zhong M, Guo S (2016). The role of 24-epibrassinolide in the regulation of photosynthetic characteristics and nitrogen metabolism of tomato seedlings under a combined low temperature and weak light stress. *Plant Physiology & Biochemistry* 107:344-353.
- Siri B, Vichitphan K, Kaewnaree P, Vichitphan S, Klanrit P (2013). Improvement of quality, membrane integrity and antioxidant systems in sweet pepper (*Capsicum annuum* Linn.) seeds affected by osmopriming. *Australian Journal of Crop Science* 7(3):2068.
- Sivritepe N, Sivritepe HO, Eris A (2003). The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. *Scientia Horticulturae* 97:229-237.
- Summart J, Thanonkeo P, Panichajakul S, Prathepha P, Mcmanus MT (2010). Effect of salt stress on growth, inorganic ion and proline accumulation in Thai aromatic rice, Khao Dawk Mali 105, callus culture. *African Journal of Biotechnology* 9:145-152.

- Tuna AL, Kaya C, Dikilitas M, Higgs D (2008). The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environmental and Experimental Botany* 62(1):1-9.
- Varier A, Vari AK, Dadlani M (2010). The subcellular basis of seed priming. *Current Science* 99:450-456.
- Xia XJ, Huang LF, Zhou YH, Mao WH, Shi K, Wu JX, ... Yu JQ (2009). Brassinosteroids promote photosynthesis and growth by enhancing activation of Rubisco and expression of photosynthetic genes in *Cucumis sativus*. *Planta* 230:1185.
- Yan QY, Duan ZQ, Li JH, Xun L, Dong JL (2013). Cucumber growth and nitrogen uptake as affected by solution temperature and $\text{NO}_3^-:\text{NH}_4^+$ ratios during the seedling. *Korean Journal of Horticultural Science & Technology* 31:393-399.
- Soeda Y, Konings MC, Vorst O, van Houwelingen AM, Stoopen GM, Maliepaard CA ... van der Geest AH (2005). Gene expression programs during *Brassica oleracea* seed maturation, osmopriming, and germination are indicators of progression of the germination process and the stress tolerance level. *Plant Physiology* 137(1):354-368.
- Yi - Fang C, Yi W, Wei - Hua W (2008). Membrane transporters for nitrogen, phosphate and potassium uptake in plants. *Journal of Integrative Plant Biology* 50:835-848.
- Zhang L, Meng XX, Liu N, Yang JH, Zhang MF (2012). Effects of grafting on phosphorus uptake and utilization of watermelon at early stage under low phosphorus stress. *Journal of Fruit Science* 29(1):120-124.



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