



Effects of hormonal priming on seed germination of pigeon pea under cadmium stress

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ABSTRACT

In this work we investigated whether priming with auxin, cytokinin, gibberellin, abscisic acid and ethylene, alters the physiological responses of seeds of pigeon pea germinated under water and cadmium stress. Seeds treated with water or non-treated seeds were used as control. Although compared to non-treated seeds we found that the hormone treatments improve the germination of pigeon pea under cadmium stress, however, these treatments did not differ from water. However, we also observed a trend of tolerance to the effects of cadmium in the presence of ethylene, suggesting that the use of this hormone may be an efficient method to overcome seed germination under metal stress.

Key words: *Cajanus Cajan*, heavy metal, hormones, pretreatment in seeds, abiotic stress.

INTRODUCTION

Cadmium (Cd) is a toxic heavy metal that has become a major pollutant due to improper agricultural and industrial activities. Low concentrations of Cd absorption can disrupt vital plant processes such as photosynthesis and nitrogen metabolism, thus adversely affect growth and productivity (Arasimowicz-Jelonek et al. 2011, Gill and Tuteja 2011, Cui et al. 2013). Additionally, Cd accumulation in crop plants poses a severe threat to human health (Jarup and Akesson 2009).

Seed priming is a technique that can be applied to improve germination and growth in heavy metal-contaminated areas. During priming, the

germination process is induced by soaking seeds in water or in solutions containing exogenous molecules such as salts (Khan et al. 2009), metals (Mirshekari et al. 2012) or hormones (Nakaune et al. 2012), but then halted by drying of the seeds. Primed seeds tend to show better germination and growth even when imposed to stressful conditions. Although the mechanisms on how priming improves these parameters are still unclear, it has been suggested that the strategy activates a series of physiological processes that improve plant growth under stressful conditions (Varier et al. 2010), including the induction of antioxidant systems (Eisvand et al. 2010).

Hormone pretreatment is a commonly used priming strategy to improve seed germination in

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stressful conditions (Atici et al. 2003, Gratão et al. 2005, Jisha et al. 2013, Masood et al. 2012, Hu et al. 2013). For example, seeds of rye (*Secale montanum*) pretreated with gibberellic acid (GA_3) increased germination in water deficit conditions (Ansari et al. 2013). In pepper (*Capsicum annum* L.), Khan et al. (2009) showed that pretreatment with acetylsalicylic acid and salicylic acid resulted in greater uniformity of germination and establishment of seedlings under high salinity. Additionally, ethylene was used to minimize the effect of high temperatures on seed germination of lettuce (*Lactuca sativa* L.) (Nascimento 2004). In the present study, we aimed to investigate whether hormone priming alters the physiological responses of the economically important pigeon pea (*Cajanus cajan* L. Mill sp - Fabaceae), when grown under Cd stress.

MATERIALS AND METHODS

PLANT MATERIAL AND PRIMING TREATMENTS

Pigeon pea (*Cajanus Cajan* (L.) Mill sp) seeds were soaked for 17h (25 °C, dark) in 200 mL solutions containing 10 or 100 μ M of the following hormones: Auxin (AUX- indolebutyric acid or IBA), gibberellin (GA_3), cytokinin (CK, 6-benzilaminopurine or 6-BAP), ethylene (ET, chloroethylphosphonic acid or CEPA), abscisic acid (ABA) and distilled water (hydro-priming). Non-priming seeds (NP) were taken as control. After the priming treatment, seeds were washed with distilled water and dried on filter paper at room temperature (in shade) for 24h (Bennett and Waters 1987, Khan 1992).

STRESS INDUCTION

Seeds were then sterilized for three minutes in a solution of 5% sodium hypochlorite and allowed to germinate in plastic containers containing two layers of filter paper moistened with distilled water. After four days, a solution containing 50 μ M cadmium chloride ($CdCl_2$) was added to the containers. As a

mock control, a set of seeds were left in water. The experiment was conducted in a growth chamber at 25 °C and long-day photoperiod (16h light).

GERMINATION TEST

We used four replicates of 36 seeds to calculate: (i) the percentage of seeds that germinated [$(G_i\% = (ni/N) \times 100)$]; (ii) the final germination percentage [$(G_f\% = (nf/N) \times 100)$], where ni and nf were, respectively, the number of seeds germinated every day or at the end of the whole experiment (7 days), and N was the number of seeds included in the test; (iii) the germination speed index [$GSI = \sum(ni/ti)$], where ti was day i ; (iv) the average germination time [$AGT = \sum(ti.ni) / \sum n$] and; (v) the time to reach 50% germination $\{T_{50} = t_i + [(N/2) - n_i](t_j - t_i) / (n_j - n_i)\}$, where N is the final number of seeds that germinated and n_i and n_j were the cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $ni < N/2 < nj$ (Coolbear et al. 1984, Farooq et al. 2005). Germination was defined as the visible emergence of the radicle through the seed coat (Maguire 1962, Galmés et al. 2006).

STATISTICAL ANALYSES

The data was submitted to statistical analysis by using analysis of variance (ANOVA) where means were compared between treatments by LSD (least significant difference) at the 0.05 confidence level using Tukey's test.

RESULTS AND DISCUSSION

Results of the experiment with AUX, CK, GA_3 , ABA priming and hydro-priming showed that, even under Cd stress, pigeon pea seed germination was improved after 48h as compared to NP control (Fig. 1a – 1h). Although we found higher values of $G_f\%$ in the hormone treated seeds when compared to the NP, the fact that these values did not differ from seed treated with hydro-priming indicates that H_2O itself and not the presence of AUX, CK, GA_3 in the solutions lead to the germination

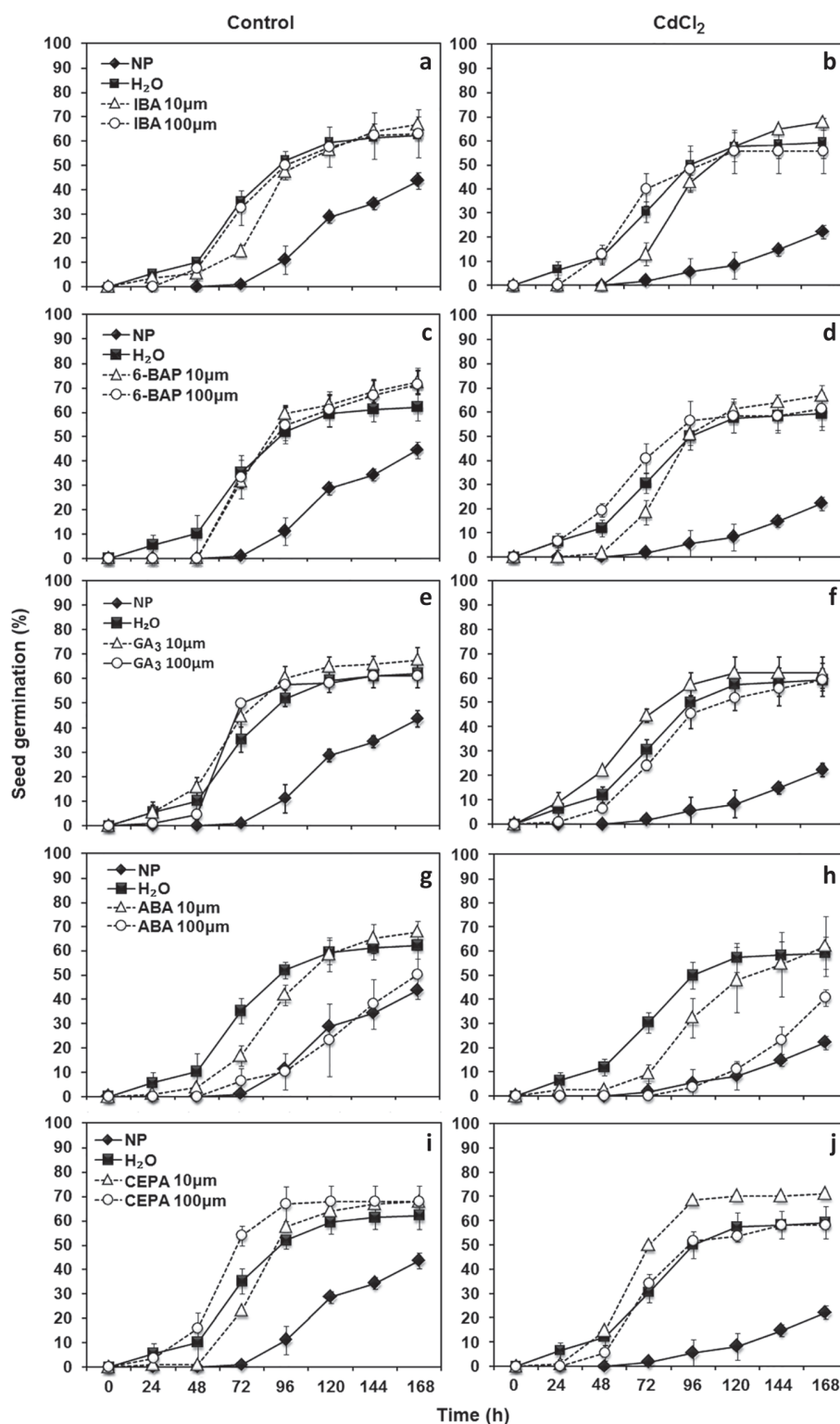


Figure 1 - Seed germination percentage at 24h intervals ($G_t\%$) of pigeon pea seeds pretreated with hormones (a-b) auxin (IBA), (c-d) cytokinin (6-BAP), (e-f) gibberellins (GA), (g-h) abscisic acid (ABA) and (i-j) ethylene (CEPA) incubated in control condition or under cadmium stress (CdCl_2) during 168h. Error bars represent $\pm\text{SE}$.

improvement (Table I). Interestingly, we found that until 120h after induction of germination, seeds treated with 100 μM ABA had similar germination rates of NP (Fig. 1g and 1h). The results obtained with the higher concentration of ABA (100 μM)

was probably related to the known inhibitory action that ABA carries on the synthesis of enzymes involved in the degradation of endosperm cell wall, an important step for seed germination (Nambara et al. 2010, Linkies and Leubner-Metzger 2012).

TABLE I
Germination speed index (GSI), average germination time (AGT), time to reach 50% germination (T_{50}) and final germination percentage ($G_f\%$) obtained from seeds of pigeon pea pretreated with the hormones auxin (IBA), cytokinin (6-BAP), gibberellins (GA_3), ethylene (CEPA) and abscisic acid (ABA) and distilled water (hydro-priming) while non-priming seeds (NP) were taken as control.

Treatment	GSI		AGT		T_{50}		$G_f\%$		
	H ₂ O	CdCl ₂	H ₂ O	CdCl ₂	H ₂ O	CdCl ₂	H ₂ O	CdCl ₂	
NP	0.13±0.01	0.06±0.01	125.97±7.43	136.00±13.68	112.21±8.66 ^a	192.05±6.11 ^b	43.52±3.34 ^a	22.22±2.78 ^b	
H ₂ O	0.33±0.06*	0.33±0.02*	80.54±9.42*	79.57±6.41*	62.68±13.03*	59.67±5.17*	62.04±5.63*	59.26±6.68*	
IBA	10 μM	0.29±0.03 ^{a*}	0.24±0.01 ^{b*} ▲	98.72±4.28 ^{a*} ▲	104.92±1.58 ^{a*} ▲	80.02±6.22 [▲]	88.54±1.57 ^{*▲}	66.67±3.21*	67.59±1.85*
	100 μM	0.28±0.03 ^{*▲}	0.28±0.05*	87.48±2.99 ^{a*} ▲	76.83±1.49 ^{b*}	67.39±5.49*	59.13±4.37*	62.96±9.80*	55.56±8.93*
6-BAP	10 μM	0.29±0.01 ^{a*}	0.27±0.02 ^{b*} ▲	93.72±4.12 ^{*▲}	99.66±1.55 ^{*▲}	77.49±2.77 [▲]	79.13±3.51 ^{*▲}	72.22±4.81*	69.44±3.21*
	100 μM	0.29±0.03*	0.37±0.02*	95.19±6.23 ^{a*} ▲	73.81±0.33 ^{b*} ▲	79.87±3.17 ^{a*} ▲	51.74±3.05 ^{b*}	71.30±6.68*	61.11±6.99*
GA_3	10 μM	0.38±0.05*	0.41±0.05*	77.08±1.30 [*]	68.21±1.01 ^{a*} ▲	55.02±5.76*	47.04±1.98*	67.59±5.16*	62.04±6.48*
	100 μM	0.31±0.02 ^{a*}	0.26±0.03 ^{b*} ▲	76.73±1.82 [*]	93.76±2.47 ^{b*} ▲	67.90±5.25*	70.56±1.85*	61.11±0.00*	59.26±4.63*
ABA	10 μM	0.27±0.02 ^{*▲}	0.24±0.05 ^{*▲}	101.61±5.53 ^{*▲}	110.27±2.59 ^{*▲}	82.74±6.48 [▲]	92.25±4.68 ^{*▲}	67.59±4.63*	62.96±11.82*
	100 μM	0.15±0.04 [▲]	0.10±0.01 [▲]	134.17±9.68 [▲]	146.24±3.90 [▲]	114.55±6.36 [▲]	127.32±3.95 ^{*▲}	50.00±10.02 ^a	40.74±3.34 ^{b*} ▲
CEPA	10 μM	0.28±0.02 ^{a*}	0.37±0.01 ^{b*}	92.49±2.96 ^{a*} ▲	75.38±1.62 ^{b*} ▲	75.83±3.44 ^{a*} ▲	57.19±1.34 ^{b*}	67.59±2.45*	71.30±2.45*
	100 μM	0.39±0.02 ^{a*}	0.27±0.02 ^{b*} ▲	69.74±3.20 ^{a*} ▲	84.28±1.79 ^{b*}	55.06±7.23*	67.94±3.76*	67.59±6.68*	58.33±1.60*

Means marked with asterisk in the column indicate that hormonal priming or H₂O are different from NP, and means marked with triangle indicates that hormonal priming is different from H₂O treatment. Different letters in the row indicate that there is significant difference between H₂O and CdCl₂. These differences are obtained from Duncan's test at $P < 0.05$.

In the case of ET, seeds pretreated with H₂O, 10 or 100 μM of the exogenous hormone showed, both in control and CdCl₂ treated seeds, higher rates of $G_f\%$, compared to NP (Fig. 1i and 1j). We found an increase in the $G_f\%$ from seeds pretreated with 10 mM of ET after 48 to 96h in the presence of Cd (Fig. 1j), suggesting that ET pretreatment lead to a decreased sensitivity or tolerance to exogenous applied Cd. However further research is still necessary to prove the role of ET priming on pigeon pea seeds since no differences were observed

for GSI, AGT, T_{50} and $G_f\%$. Plant responses to ET priming can be rather complex, it has been previously documented that seed priming with ACC (1-aminocyclopropane-1-carboxylic acid), an ethylene precursor, increased the germination rate of lettuce seeds (Nascimento et al. 2004), but not of ryegrass (Tiryaki et al. 2004).

We found that hydro- and not hormone priming is the main factor improving the germination of pigeon pea. This treatment also improves germination efficiency under high Cd concentrations and can be

used as an efficient alternative to improve seed germination in Cd contaminated areas. Hormonal effects were observed for high ABA concentrations, where we demonstrate the inhibitory action of this hormone during pre-germination period. We also observed a trend of tolerance to the effects of Cd in the presence of exogenous ET, but further studies are still necessary to prove the role of this hormone during heavy-metal tolerance.

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RESUMO

No presente trabalho nós investigamos como o pré-tratamento com auxina, citocinina, giberelina, ácido abscísico e etileno alteram as respostas fisiológicas de sementes de feijão guandu germinadas em água e sob estresse causado por excesso de cádmio. As sementes tratadas com água ou não-tratadas foram utilizadas como controle. Quando comparadas às sementes não tratadas, percebemos que os pré-tratamentos hormonais resultaram em um aumento na germinação do feijão guandu na condição de estresse. Entretanto, esses tratamentos não diferiram daqueles apenas com água. Nós também observamos uma tendência de tolerância aos efeitos do cádmio na presença de etileno, sugerindo que o uso desse hormônio possa ser um método eficiente para superar o efeito do estresse por cádmio durante a germinação de sementes.

Palavras-chave: *Cajanus Cajan*, metal pesado, hormônio, pré-tratamento de sementes, estresse abiótico.

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