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Effects of phytohormone seed priming on germination and seedling growth of cowpea (*Vigna sinensis* L.) under different duration of treatment

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Abstract

The experiment was undertaken with an objective to determine how the rate of seed germination and seedling growth can be influenced by various concentrations of growth regulators i.e. GA_3 and IAA in cowpea. Seed moisture content was determined and found optimum for seed testing. The seeds were soaked in different concentrations (20, 40, and 60 ppm) of GA_3 and IAA for 6 or 12 hours. Four replicates of each treatment with 15 seeds per replicate were arranged for precise analysis. Significant variation was found between the treatments in all aspects. Distilled water showed highest germination rate as well as the higher radicle and plumule length in contrast to other treatments. We found a drastic differences between the time soaking and the 6 hrs was more effective than 12 hrs in all aspects.

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Introduction

Cowpea (Leguminosae:Papilionoidae) represent the main food legume and a versatile crop in tropical region. It is drought tolerant and could produce better growth in warm climates. It is most popular in the semi arid regions of the tropics where other food legumes are available (Barba-Espín *et al*, 2010). The crop has been described as the major source of dietary protein in tropical and subtropical regions of the world especially where animal protein consumptions are low (Barba-Espin *et al.*, 2011). Efforts made to maximize yield, is largely hampered by adverse effect of a biotic stress such as salinity and drought. These effects cause a huge loss due to low yield and failure of the crop to establish in some cases. Alternative approach towards efficient and cost effective means of production of cowpea in the tropical regions is very desirable. Pre-sowing hardening seed treatment is an easy, low cost and low risk technique and also an alternative approach recently used to overcome the effect of abiotic stresses in agricultural production. Increased germination rate and uniformity have been attributed to metabolic repair during imbibition (Farooq *et al.*, 2007), buildup of germination enhancing metabolites (Basra *et al.*, 2005), osmotic adjustment (Bradford, 1986), and, for seeds that are not redried after treatment, a simple reduction in imbibition lag time (Bradford, 1986). The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, soybean and sunflower (Bajehbaj, 2010; Mehmet, 2006; Sadeghian and Yavari, 2004). The hardening treatment proved to be better for vigour enhancement than the traditional soaking (Basra *et al.*, 2005).Under field conditions poor seed quality not only reduces the final crop stand but also delays the onset of germination and adversely affects the seedling vigour. Seed quality is considered as a major tool of a variety development in seed production and breeding process. It is a well establish fact that, pre-soaking seeds with optimal concentration of phytohormones enhance their germination, growth and yield of some crop species under condition of environmental stress by increasing nutrient reserves through increased physiological activities and root proliferation (Radi *et al.*,2001; Afzal *et al.*,2002; Akbari *et al.*,2007). Previous studies have also shown that presowing seed treatment in various concentration of Indole acetic acid (Basra *et al.*, 2006) and Gibberellic acid (Alonso-Ramírez *et al.*, 2009; Radi *et al.*, 2001; Anguish *et al.*, 2001) may promote or inhibit seedling growth. However little is emphasized on how plant growth hormones could affect cowpea seed germination and seedlings growth. The main objective was to assess the physiological effect of Indole 3acetic acid (IAA) and Gibberallic acid (GA_3) on germination and seedling growth of cowpea.

Material and methods

This experiment was carried out at the Seed Testing Laboratory, Department of Seed Science & Technology, at the Shoushtar University in Iran, with an objective to determine the rate of seed germination and seedling growth which influenced by various concentrations of growth regulators in cowpea (*Vigna sinensis* L.). Seeds of cowpea were obtained from the International Institute of Agriculture (IITA), Safiabad Research Station., Ahvaz, Iran. Before the start of experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and air-dried. Moisture content of seed was determined by using oven at 103^{o}C for 12 hrs and was found 12% as recommended value.

Pre-sowing and planting of seeds

Different concentrations of the growth substances prepared in the laboratory were transferred from the reagent bottles into 50mls conical flasks which were clearly labeled according to the concentration of the growth substances to be used in the soaking treatment. The seeds were soaked in the various concentrations of 20, 40 and 60 ppm of GA_3 and IAA with a separate control set. These were soaked for 6 or 12 hours in the above concentrations and only double distilled water for the control set. The seeds were sown on moist filter papers in 9cm well labeled Petri dishes. Four replicates of each treatment with 15 seeds to each replicate were placed in seed germinator, 20ºC. Observation aspects like germination count (recorded for 7 days), measurement of radicle and plumule length was measured. Seed germination was recorded by skipping every two days and radicle and plumule length was measured every alternate day till the final day of experimentation. The coefficient of uniformity of emergence (CUE) was calculated using the following formulae: $CUE = n / \sum [(T - t)^2 \times n]$.

Where T is the mean germination time,'t' is the time in days, starting from day 0, and n is the number of seeds completing emergence on day t'. The radicle and plumule length of each treatment were calculated, and for quantitative evaluation of effect of various treatments, the values were used to compare with the control treatment observation values. All percentages were transformed prior to analysis. The experiment was laid out in Completely Randomized base on factorial Design (RCD) with 4 replications. Data collected were analysed statistically using coefficients of variability and least significant difference (LSD) test at 0.05 probability level (Steel and Torrie, 1984).

Results and discussion

Emergence rate, root shoot lengths, seedling biomass are all important contributors of seed vigor. Higher emergence rate is the main foundation, which ensures an improvement of overall seedling performance. Seed germination rate varied significantly among the duration and hormone treatment ($P<0.05$). The results showed significant increase in the rate of germination for seeds presoaked in the distilled water when compared with various hormones concentrations (Table 1). Maximum increase of up to about 25 and 71% in compare to presoaked GA_3 and IAA was observed. The soaking period of 12 hrs decreased the germination rate and uniformity emergence significantly in respect to 6 hrs treatment (Table 1). Substantial variation on germination and other aspects was found between treatments (Table 2). The seeds treated with GA_3 showed better performance, in compare with IAA treatments. In comparison, concentrations of GA_3 did not show any difference in respect of all measured traits which meant the higher concentration was as good as the lower concentration. Germination rate under the treatments of IAA at all concentrations recorded maximum by 12 hrs soaking (Table 2). The highest concentration of IAA (60 ppm) showed the most fresh weight (12.64 mg) however, there was not any significant differences in compare to control treatment. Observations revealed that in both the cases whether it is GA_3 or IAA, the uniformity emergence percentage decreased when the concentration increased, which shows that higher concentration retard germination (Table 2). The shortest radicle length was recorded in (IAA 20 ppm) after 12 hrs (Table 2). A longest plumule elongation was observed in the distilled water after 6 hrs (14.07 mm), and the shortest elongation was found in IAA 20 ppm (2.5 mm) after 12 hrs. Besides to germination rate, radicle or plumule length, seedling dry weight and uniformity emergence showed significant difference among treatments by presoaking duration time which meant there was great effect by the growth hormones and a marked reduction observed with increasing of time soaking (Table 2). However, this is likely due to the interference of time soaking on the phytohormones biosynthesis or due to libration of enzymes and action.

It was observed that for germination enhancement, distilled water was best suited, but in case of plumule length and uniformity emergence, did not show any significant effect with GA_3 (Table 1). When the two hormones were compared, Gibberellic acid (GA_3) was observed more effective and responsive to the regulation of radicle and plumule elongation which support the report of Chakrabarti and Mukherji (2003). The application of another plant growth regulator could increased the seed germination and other physiological activity by the reason of tolerance to the toxic particles which was found in consistent with the finding of Chakrabarti and Mukherji (2003; 2002); Hoque and Haque (2002). With the more effectiveness of low concentration of GA_3 could restore retardation in water content, this may able to tolerance to water stress. The result was considered in parallel to the findings of Das Gupta *et al.* (1994). As from the Table 2 information have shown that GA_3 more effective than the IAA in the seed physiological activity, the findings supports the report of Chakrabarti and Mukherji (2002). The role of plant growth regulators in overcoming the harmful effects on growth may be due to the change in the endogenous growth regulators (Kranner *et al.*, 2010). Although varied in seed germination and root shoot elongation by different treatments, the pre-soaking with different treatments evident that soaked seed could improve in germination and seedling establishment and this observation was found equivalent the observation of Iqbal *et al.,* (2007); Basra *et al.,* (2005).

Table 1. Effect of phytohormone priming on seed germination, seedling emergence and other growth traits of *cowpea* L. under different time of soaking.

Treatment	Germination rate (Seed/day)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Root length (mm)	Plumule length (mm)	Uniformity emergence
Time (hrs)						
6	8.42^{a}	11.12^{b}	1.72^{a}	29.41^{a}	7.74^{a}	0.77^{a}
12	5.58^{b}	11.69^{a}	1.67^{a}	30.65^{a}	6.99^{a}	0.41^{b}
LSD (p=0.05)	0.313	0.548	0.092	2.168	0.084	0.115
GA_3 (ppm)						
20	7.08^{b}	11.38^{a}	1.64^{b}	37.07^{a}	10.58^{a}	0.68^{a}
40	7.08^{b}	10.93^{a}	1.57^{b}	35.18^{a}	10.73^{a}	0.53^{a}
60	7.08^{b}	11.81^{a}	1.72^{b}	36.17^{a}	10.97^{a}	0.63^{a}
IAA (ppm)						
20	5.49^{c}	11.38^{a}	1.72^{ab}	23.3^{bc}	4.55^{bc}	0.56^{a}
40	5.93^{c}	10.93^{a}	1.82^{a}	25^{b}	5.05^{b}	0.58^{a}
60	6.09^{b}	11.81^{a}	1.81^{a}	19.58^{c}	3.05^{c}	0.54^{a}
Control	9.37^{a}	11.22^{a}	1.59^{b}	33.9^{a}	5.48^{b}	0.67^{a}
LSD (p=0.05)	0.587	1.025	0.171	4.06	1.58	0.051

The same letters in each column indicate an insignificant difference at the P=0.05 level. LSD–Least significant difference.

Table 2. Interaction effects of phytohormone priming and soaking time on seed germination, seedling emergence and growth of *cowpeas* L.

Hormone	Treatment Duration (hrs)	Con. (ppm)	Germination rate (Seed/day)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Root length (mm)	Plumule length (mm)	Uniformity emergence (%)
GA3	6	20	8.33^{b}	11.46^{abcde}	1.75^{abcd}	27.17^{e}	5.7^{efg}	0.88^{b}
		40	8.33^{b}	10.87^{de}	1.6^{cde}	30.93^{cde}	6.67^{ef}	0.83^{b}
		60	8.33^{b}	12.52^{ab}	1.86^{ab}	20.93^{f}	3.6^{ghi}	0.78^{c}
	12	20	8.33^{b}	11.3^{abcde}	1.7^{abcde}	32.33^{cde}	9.67^{cd}	0.86^{b}
		40	6.86^{c}	9.42^{f}	1.48^{e}	33.7^{cd}	12.5^{ab}	0.69^{d}
		60	6.25^{cd}	11.98^{abcd}	1.76^{abcd}	28.37^{de}	7.87^{de}	0.63^{d}
IAA	6	20	5.83^{d}	10.27^{ef}	1.54^{de}	32.43^{cde}	5.9^{ef}	0.67^{d}
		40	4.73^{e}	11.3^{abcde}	1.65^{abcde}	19.43^{f}	3.4^{hi}	0.57^{e}
		60	5^{ef}	10.98^{cde}	1.6^{cde}	19.07^{f}	3.43^{hi}	0.66^{d}
	12	20	5.83^{d}	11.1^{bcde}	1.63^{bcde}	18.33^{f}	2.5^{i}	0.67^{d}
		40	5.83^{d}	12.37^{abc}	1.83^{abc}	41.8^{ab}	11.5^{bc}	0.68^{d}
		60	5.83^{d}	12.64^{a}	1.87^{a}	36.67^{bc}	8.97^{d}	0.61^{de}
Control	6	_	12.5^{a}	11.26^{abcde}	1.64^{abcde}	43.97^{a}	14.07^{a}	0.97^{a}
	12	_	6.25^{cd}	12.17^{abcd}	1.79^{abc}	35.37^{c}	5.07^{fgh}	0.62^{de}
LSD=(0.05)			0.829	9.42	0.024	5.737	2.235	0.304

The same letters in each column indicate an insignificant difference at the P=0.05 level. LSD–Least significant difference.

Better performance of cowpea plants raised from seeds primed with distilled water might be due to the maintenance of tissue water contents, increase in antioxidant activities, and carbohydrate metabolism (Farooq *et al.*, 2008). The soaking period of 6 hrs in turn aid to the quick biochemical changes and it was found suitable for seed germination.

Conclusion

The findings of this study revealed that, cowpea germination and early seedling growth were promoted by pre-sowing hardening treatments in distilled water. The lower concentration of hormones (20ppm) was found to be more effective in inducing germination rate, total dry weight and uniformity emergence.

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