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Effect of cytokinin and gibberelin on lettuce seeds germination

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Abstract

This study was about germination of Lettuce (*Lactuca sativa*) performed in Azad Islamic university Shirvan branch and effects of cytokinin and gibberellin soaking were investigated in a Randomized Complete Block Design with factorial treatment structure in four replications. The solutions of cytokinin and gibberellin were prepared at 2, 4, 6 and 8 ppm levels and 1000 seed were soaked in solutions. The effects of applied hormones and the concentrations of hormone solutions were significant (0.01%). Soaking the seeds in 8 and 6 ppm of cytokinin solutions had the highest effect on duration of radicles growth (7.75 and 5.5 days respectively) and also using 8 ppm of cytokinin solution had the highest effect on duration of hypocotylesgrowth (14 days).

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Introduction

Lettuce (Lactuca sativa) is one of species of Compositae family. It is an annual plant that originally belongs to the Mediterranean area. 15 to 18ºC is temperature that lettuce head is growing. Germination occurs at a minimum of 5ºC. Warmand dry conditions cause to flowering and seed formation (bolting). Bolting occurs where temperatures over 20ºC are maintained day and night. Lettuce is currently an important crop which once was grown in larger quantities in the Atlantic Area. (Anonymous. 2005.) There is a level of α-galactosidase in the dry lettuce seeds that inhibit germination of imbibed seeds in darkness and following red-light irradiation and gibberellic acid exposure, increasing in some enzymes activities was observed in several hours prior to germination. (Leung and Bewley 1981) Also in some studies revealing that dormancy could be controlled by red and far-red light (Borthwick *et al.*, 1954). Some of seeds that need light for germination are called photoblastic seeds such as tomato and Arabidopsis. Light-inducible germination lets seeds which put in the ground, remain dormant until disinter. The germination of lettuce seeds is depend on light and is regulated by red and far red phytochromes. Indeed R and FR phytochromes are light receptors in plant. Red light irradiation persuades the germination in lettuce seeds and far-red irradiation that is followed by red irradiation; cancel the effect of red irradiation.(Y. Sawada *et al.* 2008)Plant hormones Gibberellins (GA), cytokinins and other phytohormons act as signalling molecules and have severe effect on plant growth and development especially they are extremely important for the regulation of seed dormancy and germination. (B. Kucera *et al.* 2005) GA_1, GA_{20} and GA_{19} are endogenous gibberellins in lettuce seeds. And phytochrome specifically regulates endogenous levels of GA_1. (T. Toyomasu *et al.* 1993) Cytokinins overcome the inhibitory effect of Abscisic acid on the germination of isolated embryos of Grand Rapids lettuce. On the other hand, little or no cytokinin-like activity seems to result from light or gibberellin treatments (M. Black. *et al.* 1974).

Researches were shown that gibberellins are produced by red radiation in photoblasic seeds consequently and dormancy of seeds is broken. And red radiation is known as an effective factor of after ripening process in dormant seeds that could be substituted by gibberellins. Cytokinins also take part in germination as an important substance but there are not any results about its effects on photoblastic seeds in darkness. So this study was done by considering the effect of gibberellin and cytokinin on germination of lettuce seeds.

Materials and methods

Seed preparing

This study was done in laboratory of Islamic Azad University-Shirvan Branch. 1000 lettuce seeds (Lactoca sativa L) were used in this study. Lettuce seeds were disinfected by Sodium hypochlorite 20% for 10 minutes and were rinsed twice by distilled water. For assurance of seed viability and dormancy of seeds the Tetrazolium test and Standard germination test was performed respectively.

Seeds treating

The solutions of Cytokinin and Gibberellin were prepared at 2, 4, 6 and 8 ppm levels in different tanks. Seeds were soaked in solutions for 24 hours. Treated seeds were cultured on water-saturated filter paper in 9 cm Petri dishes. The Petri's were placed in germinator at 18 degrees Celsius and 70% relative humidity without light. The germinated seeds were counted per day. Tank water of germinator was controlled. Humidity of filter paper was adjusted in each Petri.

The effects of Gytokinin and Gibberellin soaking were investigated in a Randomized Complete Block Design with factorial treatment structure in four replications.

Result and discussion

Seed germination

All treatments were promoted germination of seeds (0.05) that is shown in table 1. but no significant differences was observed between the levels of hormones. In this study the effective concentrations of solutions that were necessary to germinate, were higher than effective concentrations of solutions that were used in medicinal plants and vegetables (observed data). For enhancing germination and breaking seed dormancy in *T. polinum,* treatments including of several levels of Gibberellic acid were applied for seeds as soaking for 72 hours that the highest rate of germination gained by using 250 and 1500 ppm GA_3 solutions (Koocheki and Azizi, 2005). This results emphasized effects of gibberellins on germination of photoblastic seeds. The effects of various Cytokinins (kinetin, benzyladenine) were studied on germination of seeds of *Lotus corniculatus*that is a common flowering plant native to grassland temperate Eurasia that its seeds need light to germinate. All Cytokinins increased the percentage of seed germination up to twofold, depending on their kind and concentration (Nikoli *et al.* 2007). Effects of Gibberellin on breaking seed dormancy in *Achillea millefolium* was remarkable (A.tahmaseb.) Investigating effects of plant growth regulators by H. Isivand and H. Madah Arefi believe that Cytokinin and Gibberellin increase germination. Applying Gibberellic acid at 50, 100,200,400 ppm increased germination rate of Jojoba seeds (V. saffari and H. Shahsavand Also applying Gibberellin on *Pimpinella anisum*, *Hyssopus officinalis*, *Thymus vulgaris*, *Achillea melifolium* was effective in seeds germination.

Table 1. Analysis of variances for studied traits.

SOV	df	Radicle Growth	Rate of Germination	Hypocotyl Growth	Number of Normal plants	Rate of Normal plants	Duration of Radicle Growth	Duration of Hypocotyl Growth
Block	3	0.13^{ns}	2.13^{ns}	0.03^{ns}	0.15^{ns}	2.53^{ns}	0.42^{ns}	1.29^{ns}
Hormone	1	0.4^{ns}	6.4*	0.4^{ns}	1.22^{ns}	19.6^{ns}	24.02**	42.02**
Concentration	4	0.16^{ns}	2.6^{ns}	0.21^{ns}	0.6^{ns}	9.6^{ns}	5.66**	9.68**
Hormone*Concentration	4	0.08^{ns}	1.4^{ns}	0.08^{ns}	0.47^{ns}	7.6^{ns}	7.96**	11.46**
E	27	0.17	2.72	0.12	0.88	14.08	0.406	1.21
CV		1.66	1.66	1.42	3.84	3.84	15.64	10.63

Table 2. Mean comparison.

Hormones	concentration	Radicle Growth	Rate of Germination	Hypocotyl Growth	Number of Normal plants	Rate of Normal plants	Duration of Radicle Growth	Duration of Hypocotyl Growth
Cytokinin	2	25a	100a	25a	24.75a	99a	3.25cd	10.5c
	4	24.75a	99a	24.5a	23.5a	94a	3.75cd	12.25b
	6	24.5a	98a	25a	24a	96a	5.5a	14b
	8	24.75a	99a	24.75a	24.5a	98a	7.75a	8a
	sample	24.5a	89b	24.5a	24.5a	98a	4c	8e
Gibberellin	2	25a	100a	25a	24.75a	99a	3.25cd	9.5cde
	4	24.75a	99a	24.75a	24.5a	98a	3.25cd	9.5cde
	6	24.75a	99a	25a	24.75a	99a	3.25cd	9.75cd
	8	25a	100a	25a	24.5a	98a	3d	8.75de
	sample	25a	88b	25a	24.5a	98a	3.75cd	9.25cde

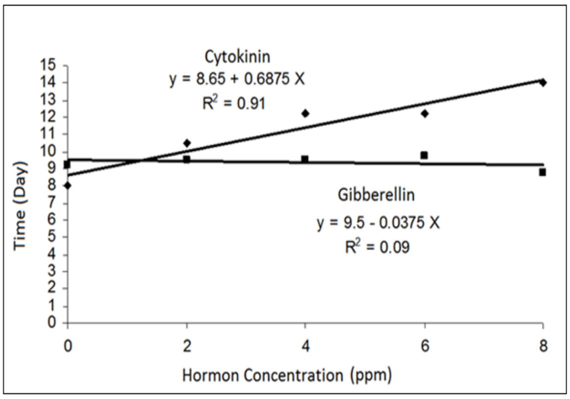

Fig. 1. Changes of duration of hypocotyls growth in different concentrations of Cytokinin and gibberellins.

Hypocotyles growth

The treatment of seeds by both of hormones was caused to increase duration of hypocotyls growth (day). Applying hormones to enhance the duration of hypocotyls growth (day) was significantly effective (0.01%). The interaction between kind of hormones and concentrations of prepared hormone solutions made significant differences in duration of radicles and hypocotyls growth (day) (0.01%). And also using 8 ppm of cytokinin solution had the highest effect on duration of growth of hypocotyles (14 days) (table 2).

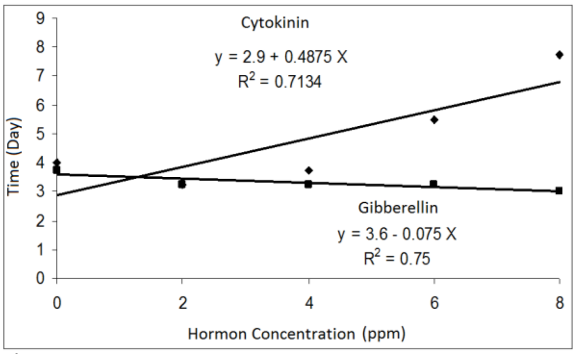

Fig. 2. Changes of duration of radicle growth in different concentrations of cytokinin and gibberellins.

Radical growth

In seeds treated by both of hormones was observed that increas duration of radicles growth (day). Also soaking the seeds in different levels of hormone solutions increased duration of radicles and hypocotyls growth (day) significantly (0.01%). That all are shown in table 1. Soaking the seeds in 8 and 6 ppm of Cytokinin solutions had the highest effect on duration of growth of radicles (7.75 and 5.5 days respectively) Homogenously-sized seeds of Tomato (S. lycopersicum) after vernalization treatments were sprayed separately by BA at 25, 50,100 mg/l, and as results a general significant increase root length, root fresh and weights, shoot length, number of leaves, number of nodes, total leaf area, shoot fresh and dry weights and relative water content was detected during tomato growth (Haroun *et al.* 2011) that there were some similarities in results in this study.

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