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Effect of Gibberllic Acid, Thiourea and Potassium Nitrate on Seed Germination and Embryo Growth of *Cupressus sempervirens* var. *pyramidalis* and *C. sempervirens* var. *horizontalis*

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Abstract

Cupressus sempervirens, the climacteric forest vegetation of El-Jable El-Akhdar (Libya), has two varieties: pyramidalis and *horizontalis*; both exhibit pronounced seed dormancy which participates in extinction of their natural vegetation. The present study investigates the effect of seed pretreatment with GA3 and thiourea at concentrations of 0, 50, 100, 200 and 400 mg/L, and with KNO3 at concentrations of 0 and 0.2% on seed germination and embryo growth of the two varieties of *C. sempervirens*. Seed germinability was higher in *horizontalis* variety than in *pyramidalis*, but rate of germination as well as embryo extension was comparable in the two varieties. Both seed germinability and embryo growth were higher in GA3 than in thiourea. Rate of germination - in terms of mean germination time - was non-significantly affected by treatments (variety, type of chemical and chemical concentration). By contrast, germinability and embryo extension increased with the increase in concentrations of GA3 and thiourea up to an optimum of 50-100 mg/L, beyond which they progressively declined. The exact optimum concentration, the magnitude of promotion at that optimum and the magnitude of retardation beyond the optimum varied according to variety and the chemical used. KNO3 at a concentration of 0.2% had limited effect on seed germination of the two varieties as well as embryo extension of *horizontalis* variety compared with that of GA3 and thiourea but increased embryo extension of *pyramidalis* variety to more than twice the control.

Keywords: Seed dormancy; *Cupressus*; GA3, thiourea, potassium nitrate.

Introduction

Seeds of many wild plants are dormant when first shed and will not sprout even when exposed to adequate moisture and oxygen supplies and appropriate temperature (Salisbury and Ross, 1978). This phenomenon is due to several causes including the hard seed coat which restricts embryo expansion, impermeability of seed coat to water and gases and the immature embryo. Many practices have been used to break seed dormancy among which is dipping seeds in sulphuric acid of different concentrations to soften the hared seed coat (Hadad, 1995) and treatment with the plant hormone gibberellic acid (GA_3) (Abdalla & Mckelvie, 1980), thiourea (Ibrahim & Haikel, 1991) and potassium nitrate (ISTA, 1993).

In Libya, El-Jable El-Akhdar has unique plant cover of many wild tree species in the form of frosts which in turn provide the appropriated micro-environment to numerous understory herbs and shrubs. *Cupressus* species are among the most important frost trees native to this region where they cover a large area of the land (Sherif and El-Taife, 1986). The natural vegetation of this area is suffering from undue practices including overgrazing, trampling, fires, over cutting and uprooting of plants (El-Barasi and Buhwarish, 2005). Seeds of *Cupressus sempervirens* L. var *horizontalis* and var. *pyramidalis* exhibit pronounced dormancy which may lead to limitation and deterioration of the natural vegetation of these varieties (El-Bakkosh, 2001). The present study investigates the efficiency of different treatments in breaking seed dormancy and hastening seedling growth of two varieties of *Cupressus* grown naturally in El-Jable El-Akhdar (Libya).

Materials and methods

The seeds of two Cupressus varieties were collected randomly from different sectors of El-Jable El-Akhdar (Libya). The seeds were dipped in tap water to eliminate shriveled seeds. A random sample of 100 seeds of each variety was subjected to the teterazolium seed viability test of ISTA (1993). Seeds were sterilized with 10% sodium hypochlorite for 5 min followed by washing with distilled water. The seeds were then dipped in concentrated H2SO4 for 10 min, washed thoroughly and blotted dry. The seeds were then soaked in different concentrations (0, 50, 100, 200 and 400 mg/L) of GA3 and thiourea and in 0.2% KNO3 for 24 h. After soaking, seeds were germinated in Petri dishes lined with filter papers moistened with distilled water (20 seed / dish) in a growth chamber at 30ºC/12 h light and 20ºC/12 darkness.

Number of germinated seeds was recorded frequently for a period of 5 weeks. Seeds were considered germinated when the radicle emerged to a length of 2 mm. The final germination percentage and the mean germination time (MGT) were calculated according to Rawal et al. (1998). The lengths of radicles and plumules were recorded.

The experiment was factorial with three main factors and four replications in a completely randomized design (CRD). The main factors were plant variety, type of pre-treatment chemical and chemical concentration. Final germination percentage was arcsine transformed before doing ANOVA to ensure homogeneity of variance. ANOVA was performed using SPSS program version 22. Mean separation was done according to the Tukey's test.

Results

Table 1 shows the effects of main factors (variety, type of chemical and chemical concentration) on germination parameters and embryo growth of *Cupressus sempervirens*. The germination parameters estimated were germinability in terms of the cumulative final germination percentage and rate of germination in terms of mean germination time (MGT), which is an inverse measure of rate of germination. Embryo growth was estimated in terms of lengths of radicle and plumule. Treatments substantially (very highly significantly, $p < 0.001$) affected magnitude of germination but had relatively limited effect on rate of germination; where MGT was non-significantly affected by variety and type of chemical but highly significantly affected by concentration only. Embryo growth was comparable in the two varieties but was very highly significantly affected by type of chemical and chemical concentration.

Table 1 Three-way ANOVA of the effects of main factors (variety, type of chemical and chemical concentration) on germination parameters and embryo growth of *Cupressus sempervirens.*

Source of variation	df	F	P	F	P
		Final germination (deg.)		Plumule length	
Variety	1	27.10	0.000	0.007	0.934
Chemical	1	11.94	0.001	142.6	0.000
Concentration	4	17.38	0.000	48.46	0.000
		Mean germination time		Radicle length	
Variety	1	0.000	1.000	0.933	0.338
Chemical	1	0.422	0.518	152.5	0.000
Concentration	4	5.689	0.001	47.29	0.000

Seed germinability was in general higher in *horizontalis* variety of *Cupressus* than in *pyramidalis* variety; and in both varieties it was higher in GA_3 than in thiourea (Tables 2 and 3). Germinability of the two *Cupressus* varieties increased with the increase in pretreatment concentration of GA_3 and thiourea up to a certain level, beyond which germinability progressively decreased with further increase in concentration up to 400 mg/L. The optimum concentration, the magnitude of promotion of germination at that optimum and the magnitude of retardation beyond the optimum concentration varied according to variety and the chemical used. Germinability increased by 56% and 72% as GA_3 concentration increased from 0 to 100 mg/L and from 0 to 50 mg/L in *horizontalis* and *pyramidalis* varieties respectively; and this was followed by 48% and 73% reductions respectively upon further increase in GA_3 concentration beyond these optima up to 400 mg/L. By contrast, germinability increased by 26% and 4% with the increase in thiourea concentration from 0 to 50 mg/L and from 0 to 100 mg/L in *horizontalis* and *pyramidalis* varieties respectively; with subsequent reductions of 36% and 66% respectively upon further increase in thiourea concentration beyond these optima up to 400 mg/L. Pretreatment of seeds with 0.2% KNO_3 (2000 mg/L) increased gerninability by 21% and 32% above the control in *horizontalis* and *pyramidalis* varieties respectively. In contrast to their marked effect on germinability, treatments had non-significant effect on mean germination time of the two varieties and the MGT averaged around 7.2 days irrespective of the variety and type and concentration of the chemical.

Table 2. Effect of gibberelic acid (GA_3), thiourea and potassium nitrate on mean germination time (MGT) and seed germinability (final germination %) of *Cupressus._sempervirens* var *horizontalis*. Each value is the mean of 4 replicates ± SE

Treatment	**Mean Germination Time (day)**	**Final germination %**
Control	7.30 ± 0.48[a]	25.67 ± 2.45[a]
GA_3 (mg/L)		
50	7.30 ± 0.47[a]	35.00 ± 3.84[b]
100	7.30 ± 0.48[a]	40.17 ± 4.49[bc]
200	7.30 ± 0.50[a]	30.33 ± 3.23[c]
400	7.30 ± 0.55[a]	21.00 ± 2.52[e]
Thiourea (mg/L)		
50	7.10 ± 0.49[a]	32.50 ± 3.02[a]
100	7.10 ± 0.49[a]	31.01 ± 3.16[ab]
200	7.10 ± 0.47[a]	30.17 ± 2.84[abc]
400	7.10 ± 0.55[a]	20.83 ± 2.50[d]
0.2 % KNO_3	7.30 ± 0.51[a]	31.00 ± 3.40

Table 3. Effect of gibberellic acid (GA_3), thiourea and potassium nitrate on mean germination time (MGT) and seed germinability (final germination %) of *Cupressus._sempervirens* var *pyramidalis***.** Each value is the mean of 4 replicates ± SE.

Treatment	Mean Germination Time (day)	Final germination %
Control	7.30 ± 0.63[a]	22.83 ± 2.80[bce]
GA_3 (mg/L)		
50	7.30 ± 0.47[a]	39.33 ± 2.27[a]
100	7.30 ± 0.48[a]	33.17 ± 3.89[ab]
200	7.30 ± 0.49[a]	22.67 ± 2.78[c]
400	7.30 ± 0.65[a]	10.83 ± 1.66[d]
Thiourea (mg/L)		
50	7.10 ± 0.55[a]	23.33 ± 2.89[ab]
100	7.10 ± 0.51[a]	23.83 ± 3.0[a]
200	7.10 ± 0.56[a]	17.83 ± 2.3[abc]
400	7.10 ± 0.90[a]	8.17 ± 1.40[d]
0.2 % KNO_3	7.10 ± 0.54[a]	30.17 ± 3.32

Embryo extension was comparable in the two *Cupressus* varieties but was improved by application of chemicals where the effect of GA_3 pretreatment was greater than that of thiourea (Tables 4 and 5). Increasing GA_3 concentration from 0 to 100 mg/L increased lengths of plumule and radicle of *horizontalis* variety by an average of 4 fold, followed by a sharp reduction of 98% upon further increase in GA_3 concentration from 100 to 400 mg/L. In *pyramidalis* variety increasing GA_3 concentration from 0 to 50 mg/L increased lengths of plumule and radicle by about 3 fold, followed by a sharp reduction of 90% upon further increase in GA_3 concentration from 50 to 400 mg/L. Increasing thiourea concentration from 0 to 100 mg/L increased lengths of plumule and radicle of *horizontalis* variety by an average of 58%, but further increase in thiourea concentration from 100 to 400 mg/L reduced lengths of plumule and radicle by 82% and 68% respectively. In *pyramidalis* variety lengths of plumule and radicle were progressively reduced by 89% and 80% respectively with the increase in thiourea concentration from 0 to 400 mg/L. Pretreatment with 0.2% KNO_3 did not significantly affect embryo extension of *horizontalis* variety but increase lengths of plumule and radicle of *pyramidalis* variety to more than twice the control value.

Table 4. Effect of gibberellic acid, thiourea and potassium nitrate on plumule and radicle lengths of *C. sempervirens* var. *horizontalis*. Each value is the mean of 4 replicates ± SE.

Treatment	**Plumule length (mm)**	**Radicle length (mm)**
Control	3.41± 0.57[ce]	4.29 ± 0.66[cd]
GA_3 (mg/L)		
50	10.72 ± 1.14[a]	11.87 ± 1.18[b]
100	17.70 ± 1.90[b]	23.01 ± 2.40[a]
200	5.72 ± 0.82[c]	7.58 ± 0.98[bc]
400	1.71 ± 0.36[de]	2.86 ± 0.47[d]
Thiourea (mg/L)		
50	4.67 ± 0.73[ce]	5.56 ± 0.76[cd]
100	5.40 ± 0.75[cd]	6.85 ± 0.88[cd]
200	2.15 ± 0.34[ce]	3.60 ± 0.44[cd]
400	1.02 ± 0.23[e]	2.19 ± 0.36[d]
0.2 % KNO_3	3.06 ± 0.52[cde]	4.90 ± 0.69[cd]

Table 5. Effect of gibberellic acid, thiourea and potassium nitrate on plumule and raicle lengths of *C. sempervirens* var *pyramidalis*. Each value is the mean of 4 replicates ± SE.

Treatment	Plumule length (mm)	Radicle length (mm)
Control	4.99 ± 0.71[c]	5.72 ± 0.80[c]

GA_3 (mg/L)		
50	18.18 ± 2.10[a]	24.10 ± 2.87[a]
100	12.03 ± 1.49[b]	13.11 ± 1.57[b]
200	4.44 ± 0.68[c]	5.39 ± 0.76[c]
400	1.85 ± 0.36[c]	2.23 ± 0.39[c]
Thiourea (mg/L)		
50	3.87 ± 1.17[c]	3.45 ± 0.46[c]
100	2.75 ± 0.42[c]	3.76 ± 0.49[c]
200	1.91 ± 0.34[c]	2.71 ± 0.43[c]
400	0.55 ± 0.17[c]	1.15 ± 0.25[c]
0.2 % KNO_3	11.42 ± 1.57[b]	15.42 ± 2.17[b]

Discussion

The low seed germinability of the two *Cupressus sempervirens* varieties (only 24%) for native seeds can be primarily due to physiological seed dormancy and partially to mechanical restriction of the hard seed coat. Seed dormancy of the two varieties of *Cupressus sempervirens* which grow well in El-Jable El-Akhdar (Libya). Because of the importance of these trees that subjected to extinction in its natural habitat, like many other trees grown in the green mountain. The positive effect of GA_3 is mainly due to its role in stimulating the conversion of complex reserves in the seeds to sucrose, mobile amino acids and amides as well as to limiting the inhibitory effect of ABA (Devlin, 1975; Salisbury and Ross, 1987). It overcomes seed dormancy in many plants and can act as a substitute for low temperature, long day or red light (Abu-Zeid, 1990). Nevertheless, the effect of GA3 and thiourea seems to be dose-dependent. The present work revealed marked promotion of seed germinability and embryo extension by low levels of the two chemicals (50-100 mglL) with sharp inhibitory effect at higher levels. The effect of GA3 was more pronounced than that of thiourea. These results are in close agreement with those of Nasroun and Al-Mana (1992), Laura et al. (1998) and Rawal et al. (1998). Such a dose-dependent effect of the two compounds has been reported by Waali (1990) where they act as growth stimulators at a certain level beyond which their retarding effect becomes evident. The present results suggest that seed germinability is affected by genotype where it was in general higher in *horizontalis* variety of *Cupressus* than in *pyramidalis* variety and that the effect of treatments on embryo extension was by far greater than on mere emergence of the embryo (seed germination), with a limited effect on rate of germination estimated as MGT.

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الملخص العربي

عنوان البحث: أثيـر المعاملـة بحمـض الجبر يليـك ، الثيوريـا و نتـرات البوتاسيوم علـي اسـتنبات و نمــو بــذور أشـجار السـرو الأفقـي و السرو العمودى

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تمثل أشجار السـرو *Cupressus sempervirens* بسلالتيه السرو الأفقي والسرو العمـودي ذروة الغطاء النباتى بمنطقـة الجبـل الأخضـر- ليبيا. تبدى بذور كلتا السلالتين كمونا ملحوظا مما يسهم فى تزايـد تعرضهـا لخطـر الانـقراض. فى هذا البحث تم دراسة تأثيـر تركيزات متدرجة (50 – 400 ملجم/لتر) مـن مادتين منشطتين للانبات وهما منظـم النمـو حمـض الجبريليـك و الثيوريـا، بالاضافة الى نتـرات البوتاسيـوم عند تركيز 0.2% علـي إنبـات البـذور ونمو الجنين للسرو الأفقى والسرو العمودى. بينما كانت نسبة الانبات أعلى فى السرو الأفقى عن السرو العمودى كان كل من سرعة الانبات نمو الجنين متقاربا فى السلالتين. وعلى العموم كان تأثير المعاملات (السلالة ونوع المادة المستخدمة وتركيزها) محدودا على سرعة الانبات. على العكس من ذلك زادت نسبة الانبات ونمو الجنين بزيادة تركيز كل من حمـض الجبريليـك و الثيوريـا حتى تركيز أمثل تراوح بين 50 – 100 ملجم/لتر أعقب ذلك انخفاض واضح عند التركيزات الأعلى من ذلك. وتفاوتت القيمة الفعلية للتركيز الأمثل ومقدار الزيادة عند هذا التركيز والانخفاض التالى عند التركيزات العالية حسب السلالة ونوع المادة المستخدمة. كان تأثير نترات البوتاسيوم بتركيز 0.2% على انبات بذور السلالتين وكذلك على نمو جنين السلالة الافقية محدودا مقارنة بتأثير كل من حمـض الجبريليـك و الثيوريـا ولكنه زاد من نمو الجنين فى السلالة العمودية الى أكثر من الضعف.