65 години
Аграрен университет - Пловдив, Научни трудове, т. LV, кн. 1, 2010 г.
Юбилейна научна конференция с международно участие
Традиции и предизвикателства пред аграрното образование, наука и бизнес
Agricultural University - Plovdiv, Scientific Works, vol. LV, book 1, 2010
Jubilee Scientific Conference with International Participation
Traditions and Challenges of Agricultural Education, Science and Business

INFLUENCE OF GROWTH REGULATORS (IAA AND GA3) AND CHILLING TREATMENT ON PROPAGATION OF *GINKGO BILOBA* BY SEEDS

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Abstract

Seeds of *Ginkgo biloba* treated with GA3 and/or IAA at different concentrations (1000, 1500, 2000 and 2500 ppm) attained the highest germination percentage (86.33%) when treated with GA3 at 2500 ppm, whilst seedling growth was stimulated in significantly higher extends using IAA with or without GA3. Furthermore, the efficacy of chilling was separately investigated at 5°C for 1-, 2- or 3-month periods comparing to room temperature which resulted in the highest germination % (64.44%).

Key words *Ginkgo*, GA3, IAA, Auxins, Germination, Stratification, Propagation

INTRODUCTION

The remarkable interest in *Ginkgo biloba* extensively noticed lately is not fortuitous, since *Ginkgo* tree has been of interest to mankind for more than 2000 years (Van Beek, 2000). This living fossil dated back to more than 180 million years ago (Tredici, 2000) has been used for more than 3000 years in China, its natural habitat, for food, medicine or ornamental purposes. However, this interest has been intensified after EGb 761, the *Ginkgo* leaf extract, was registered for human use in 1974 in France (DeFeudis, 1998), and ever since *Ginkgo*, extraordinarily, has been a subject of a considerable amount of research; meetings, books and articles reaching about one article per day in 1997 (Tredici, 2000).

So, to meet the growing demand for *G. biloba* trees and leaves, two thousand tonnes of leaves are used each year in the world (Masood, 1997), it is necessary to accelerate the propagation system. Vegetative propagation by cutting, grafting or even basal chichi is principally employed for *Ginkgo* (Santamour *et al.*, 1983). However, quicker process and faster vegetative development of the offspring could be guaranteed using sexual propagation (Laurain, 2000).

Owing rudimentary embryos and consequently low germinability (Tommasi *et al.*, 1999), seeds of *Ginkgo* must undergo a stratification period to 30-60 days at high temperature could be followed by cold stratification for the same period (Shepperd, 2008), otherwise most of such seeds will eventually decompose, (West

et al., 1970). According to West *et al.* (1970), stratification and gibberellin metabolism are related in the germination of *Ginkgo* seeds, and exogenous GA3 would largely substitute for stratification. The present investigation was therefore devoted to the work on gibberellin and auxins as pre-germination treatment so as to bypass stratification and to achieve higher germination rate and better seedling growth.

MATERIALS AND METHODS

Treatment with IAA and/or GA3

Dispersed seeds were gathered from the ground under a *Ginkgo* tree located in the Old City, Plovdiv, Bulgaria. The outer fleshy coat (sarcotesta) was removed and the seeds were briefly washed in water with a mild detergent, then air dried at room temperature and stored at 1-5°C in paper bags until they were used.

During the end of February to the beginning of March, seeds were soaked completely in 1000, 1500, 2000 and 2500 ppm solutions of gibberellic acid (GA3), indole-3-acetic acid (IAA) and their combination IAA+GA3 for 24 h at room temperature. Seeds soaked in water served as a control. The imbibed seeds were sown immediately in a 1:1 peat-perlite mixture, in a randomized complete block design with four replications of 25 seeds per experimental unit. The experiment was performed under open air conditions at the Stat Forestry Nursery, Plovdiv. Germination percentage was determined 45 days after sowing and shoot length (cm), number of leaves, mean of leaf area (cm^2) and volume of root system (cm^3) were recorded on seven-month-old seedlings. The same procedures were repeated under the same aforementioned conditions for three successive seasons of 2008, 2009 and 2010. The results were subjected to analysis of variance (ANOVA) and differences among means were tested by Duncan's Multiple Range Test (DMRT).

Chilling treatment

The present experiment was conducted at The Biotechnology Lab.y, Fruit-Growing Institute, Plovdiv. Seeds used in this experiment were collected and cleaned as above. Seeds were surface sterilized by being first given a 2-minute fungicide treatment followed by 1-h rinsing in running water. Afterwards, seven minutes treatment with 5% Calcium hypochlorite ($Ca(ClO)_2$) was given, followed three washes with sterilized distilled water, 10 minutes each. Under aseptic condition, seeds were placed in dampened-perlite-filled jars (5 seed/jar). Chilling treatments tested were 1, 2 and 3 months at 5°C, in addition to the control (at room temperature). Three jars were assigned for each treatment. Accumulative percentage of germination was recorded at the end of each corresponding period. In the end of the three-month period, all germinated or un-germinated seeds grown in a peat-perlite (1:1) mixture. Thereafter, seedlings were transplanted and transferred to greenhouse conditions.

RESULTS AND DISCUSSION

Treatment with IAA and/or GA3

The data presented in Table 1. and Fig 1 show significant differences among different concentrations of IAA and/or GA3 concerning all the recorded traits, except for mean leaf area. Treating seeds with GA3 alone or in combination with IAA resulted in higher germination percentage comparing to IAA-treated or untreated seeds (control). There is a tendency for germination percentage to increase as the concentration of GA3 and/or IAA was increased, reaching its highest values (86.33, 85.83,) when the highest levels of GA3 (2500, 2000, respectively) were used.

Hypothesized to play a key role in the promotion of germination, GA3 had been expected to increase percentage of germinated seeds. Meanwhile, under the condition of treating intact seed, the impermeability of the seed coat could inhibit the promotive effect of GA3 on seed germination as previously reported by Johnson and Wickliff (1974). However, West *et al.* (1970) found that the application of GA3 to nonstratified *Ginkgo* seeds enhanced germination to a level that approached that of stratified ones.

Table 1. Effect of imbibition in IAA and/or GA3 at 1000, 1500, 2000 and 2500 ppm on seed germination percentage (G%) and seedling growth of *G. biloba*.

Treatment	ppm	G %	Shoot length cm	No. leaves	Mean leaf area cm^2	Total leaf area cm^2	Vol. root system cm^3
Control		68.08 ^{g}	9.06 ^{d}	2.42 ^{f}	3.45 ^{ns}	8.35	1.73 ^{e}
IAA	1000	68.67 ^{fg}	9.80 ^{bvd}	2.64 ^{f}	3.45	9.11	1.81 ^{de}
	1500	70.42 ^{efg}	13.12 ^{b}	2.97 ^{e}	3.44	10.22	2.14 ^{bcd}
	2000	71.67 ^{def}	17.01 ^{a}	3.49 ^{c}	3.46	12.08	2.44 ^{bc}
	2500	73.25 ^{de}	18.72 ^{a}	4.58 ^{a}	3.58	16.40	3.07 ^{a}
GA3	1000	74.67 ^{cd}	9.67 ^{cd}	3.10 ^{de}	3.46	10.73	2.01 ^{de}
	1500	77.33 ^{c}	10.15 ^{bcd}	3.26 ^{cde}	3.55	11.57	2.09 ^{cde}
	2000	85.83 ^{a}	10.22 ^{bcd}	3.30 ^{cd}	3.77	12.44	2.04 ^{cde}
	2500	86.33 ^{a}	10.91 ^{bcd}	3.48 ^{c}	3.60	12.53	2.16 ^{bcd}
IAA+GA3	1000	77.83 ^{c}	11..20 ^{bcd}	3.25 ^{cde}	3.59	11.67	1.99 ^{de}
	1500	77.08 ^{c}	12.78 ^{bc}	3.41 ^{cd}	3.56	12.14	2.19 ^{bcd}
	2000	82.00 ^{b}	13.12 ^{b}	3.57 ^{c}	3.64	12.99	2.52 ^{b}
	2500	81.92 ^{b}	18.40 ^{a}	4.23 ^{b}	3.58	15.14	2.90 ^{a}

Mean separation by DMRT Test , $P \leq 0.05$, ns≡ Not significant at $P \leq 0.05$

Although, IAA alone or in combination with GA3 was inferior to GA3 alone in terms of germination percentage, attained seedlings characterized with significantly taller shoots, higher number of leaves and volume of root system, in particular with the highest level of IAA (2500 ppm) alone or in combination with GA3 at the same level. This superiority is reflected by the noticeable increment in both of total leaf area and volume of root system. Post-germination poromotive effect of IAA is attributable to the roll of auxins in development and formation of the shoot and root

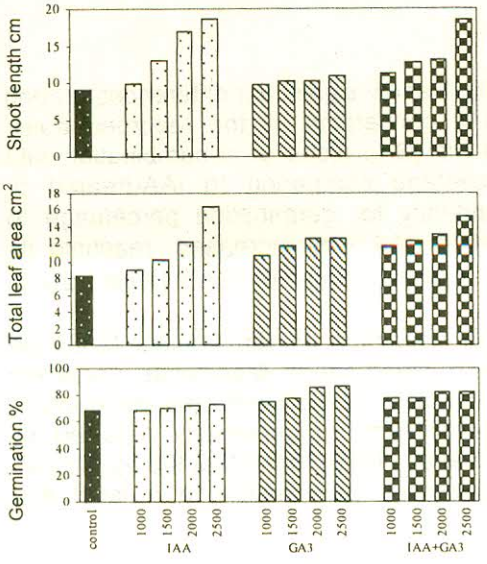

Figure 1. Effect of imbibition in IAA and/or GA3 at 1000, 1500, 2000 and 2500 ppm on seed germination % and seedling growth of *G. biloba*.

apical meristems. Although, a link has lately been drawn between exogenous auxin and embryo germinability, there is still no compelling evidence to suggest that auxin is involved in dormancy maintenance or termination per se. (Feurtado and Kermode, 2007 and references therein).

The un-germinated fraction noticed in all the treatments in the present experiment could be attributed to the presence of non-embryo-containing dispersal seeds. *Ginkgo* is one of some species whose seeds contain underdeveloped embryos, even fertilization and embryo differentiation may be delayed until after dispersal (Eames, 1955; Lee, 1955; Schmidt, 2000).

The positive effect of GA3 on seed germination has been also reported on other woody species; *Cassia obtusifolia* (Singh and Murthy, 1987), *Morus nigra* (Koyuncu, 2005), *Abies pindrow, Cupressus torulosa and Picea smithiana* (Rawat *et al.* 2006), *Pyracantha crenulata* (Joshi *et al.*, 2010), as has been IAA and IBA on *Picea smithiana* (Singh 1990), low concentration of IAA (200ppm) on *Azadirachta indica* accompanied by enhancement in seedling growth compared to GA3 application (Kumaran *et al.*, 1994).

Chilling treatment

The influence of pre-germination chilling at different periods (1, 2 and months) on seed germination comparing to non-chilling-treated seeds is illustrated in Fig. 2.Of the tested treatments, keeping seeds at room temperature (control) without any chilling treatment resulted in the highest germination percentage (64.44). A decline was noticeable as the chilling period was increased, where the longest period (3-month) had no germinated seeds. These results are in contrast to those reported by Johnson and Wickliff (1974) and West *et al.* (1970) who reported that a period of cold treatment had accelerated seed germination in *Ginkgo*, even though they found that twelve weeks of continuous cold treatment gave a slower germination rate.

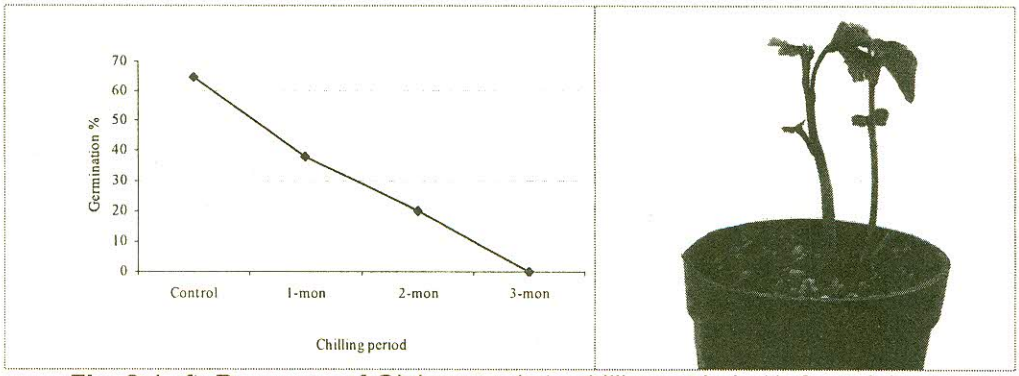

Fig. 2. Left: Response of *Ginkgo* seeds to chilling periods (1, 2, and three months) in addition to the control (at room temperature), in terms of germination percentage. Right: Twin *Ginkgo* plants resulted from stratified-seed at room temperature.

CONCLUSIONS AND FUTURE PROSPECTIVE

Considering the real need for high germination rate along with vigorous progeny, we suspect that using a combination of IAA and GA3 at 2500 ppm each could fulfill these demands. Although, stratification effects weren't as high as that obtained with the application of exogenous hormones, it could be beneficial in future investigations in increasing the permeability of *Ginkgo* seed coat and , in turn, enhancing the effects of the exogenous hormonal application.

ACKNOWLEDGEMENT

This research has been supported by Erasmus Mundus External Cooperation Window (EMECW) programme, funded by the European commission, in cooperation with Agricultural University, Plovdiv, Bulgaria. The authors express sincerest appreciation to krasimiz the manager of the State Forestry Nursery, Plovdiv for his assistance with this research.

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