Progesterone and β-Estradiol Stimulate Seed Germination in Chickpea by Causing Important Changes in Biochemical Parameters

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Effects of progesterone and β-estradiol on morphologic (germination velocity, root and shoot length) and biochemical (activities of α-amylase, superoxide dismutase, peroxidase and catalase, H_2O_2 content, lipid peroxidation) parameters during germination and post-germination stages of chickpea seeds were studied. The seeds germinated at various hormone concentrations (10^{-4}, 10^{-6}, 10^{-9}, 10^{-12}, 10^{-15} M) were harvested at the end of the 1st, 3rd, and 5th day. With comparison to the control, these hormones caused an increment in the number of germinating seeds at the end of days 1 and 3 by accelerating the seed germination. Root and shoot lengths were augmented by both hormones at all hormone concentrations tested. The highest elongation was recorded in 10^{-6} M progesterone and 10^{-9}–10^{-12} M β-estradiol. Similarly, activities of α-amylase and superoxide dismutase were increased by all concentrations of both hormones, and maximum increases were obtained with 10^{-6} M progesterone and 10^{-9}–10^{-12} M β-estradiol. In the case of superoxide dismutase activity, not only the H_2O_2 content but also the peroxidase and catalase activities increased. Lipid peroxidation decreased depending on an increase in the antioxidant enzyme activities. In the present study, it was demonstrated that progesterone and β-estradiol even at low concentrations increase the germination velocity and resistance to stress conditions by changing the activities of some biochemical pathways.

Key words: Chickpea, Seed Germination, Biochemical Activity

Introduction

Steroids are lipophilic and low molecular weight compounds. These compounds, derived from isoprenoids, have important effects on differentiation, development, and homeostasis of higher eukaryotes (Kliewer *et al.*, 1998). They act in various roles like cell membrane constituents and bioregulators such as chemical messengers, vitamins, cytotoxins, and hormones (Barrington, 1979; Sandor and Mehdi, 1979). Steroid molecules have been identified as essential growth regulators in plants as well as in animals using many analytical tools (radioimmunoassay, thin-layer chromatography, mass spectrometry, ultra-performance liquid chromatography, high-performance liquid chromatography) (Geuns, 1978; Jones and Roddick, 1988; Simons and Grinwich, 1989; Janeczko and Skoczowski, 2005; Simersky *et al.*, 2009).

Mammalian sex hormones are a member of steroids and are naturally present in plants (Sandor and Mehdi, 1979; Heftman *et al.*, 1965; Mordue and Stone, 1979; Geuns, 1978, 1982). They were first detected in plants by Dohrn *et al.* (1926) and Skarzynski (1933). Since then, many researches about the effects of these steroid hormones on growth and development of plants have been made. At the end of the studies, it was determined that steroid sex hormones applied exogenously (*e.g.* progesterone, estrone, β-estradiol, testosterone) stimulate growth and development (callus proliferation, cell division, root and shoot elongation, pollen germination flowering) (Kopcewicz, 1970; Geuns, 1978; Bhattacharya and Gupta, 1981; Shore *et al.*, 1992; Ylstra *et al.*, 1995; Hayat *et al.*, 2001; Janeczko and Skoczowski, 2005). However, there are a few reports on the effects of these hormones on seed germination. Seed germination is one of the most critical phases of plant life (Misra and Dwivedi, 2004) regulated by hormonal interactions and environmental factors (Paleg, 1960; Iglesias and Babiano, 1997; Atici *et al.*, 2005). Because there is not enough research

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and knowledge on the effects of these hormones in seed germination, it is necessary to achieve advanced researches on biochemical parameters. The present studies about effects of mammalian sex hormones on seed germination demonstrated that these hormones positively affect some morphologic (root and shoot length, percentage of germination) and biochemical parameters (protein content, amylase and catalase activity) in germinating seeds. In previous studies, however, relatively high hormone concentrations (more than 10^{-9} M) had been tested and only a few parameters had been studied (Martínez-Honduvilla *et al*., 1976; Dogra and Thukral, 1991; Dogra and Kaur, 1994; Helmkamp and Bonner, 1952).

Hence, in the present study we aimed to search the effect of progesterone and β-estradiol on morphologic (germination velocity, root and shoot length) and biochemical (activities of α-amylase, peroxidase and catalase) parameters in chickpea seeds. To the best of our knowledge, there has not been any report on the effect of mammalian sex hormones on germination in chickpea seeds, one of the most important legume crops of the world. Besides, the effects of these hormones on H_2O_2 content, lipid peroxidation and superoxide dismutase activity in germinating chickpea seeds were studied for the first time.

Material and Methods

Plant culture and steroid treatment

Chickpea seeds *(Cicer arietinum* cv. Aziziye-94) were immersed in 1% sodium hypochlorite solution for 20 min at room temperature (Aisien *et al.,* 1983) and rinsed four times into sterile distilled water. All further manipulations were carried out under sterile conditions. The steroids progesterone and β-estradiol were dissolved first in a small volume of methanol (Kato-Noguchi and Macias, 2005) and then diluted in water in order to obtain the following concentrations: 10^{-4}, 10^{-6}, 10^{-9}, 10^{-12}, and 10^{-15} M. Seeds were soaked in prepared solutions for about 6 h (Marero *et al*., 1988) and then moved in Petri dishes on filter paper moistened with 10 ml distilled water (10 seeds per dish). Seeds were soaked in distilled water, which included a small volume of methanol; they were identified as control group. All seeds were germinated in the dark at 25 ºC. The seeds germinated were counted at the 1st, 3rd, and 5th day in order to determine the percentage of germination. Root and shoot lengths were measured on the 1st, 3nd and 5th day. Steroids used in this work were obtained from Sigma-Aldrich Co., St. Louis, MO, USA.

α-Amylase activity

For determining the α-amylase activity, endosperms of seeds were cut out at the end of the 1st, 3rd, and 5th days. Crude extracts of these seeds were prepared according to Juliano and Varner (1969). The α-amylase activity was spectrophotometrically measured according to de Morais and Takaki (1998). One unit (U) of enzyme was considered as the quantity that caused an alteration of 0.1 in absorbance.

Antioxidant enzyme activity (POX, CAT, SOD)

For determining the peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) enzyme activities, seeds with germinating plant were harvested at the end of the 1st, 3rd, and 5th day. Seeds (500 mg) were homogenized in 5 ml 10 mM potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12,000 × *g* for 30 min at 4 ºC, and the supernatant obtained was used as an enzyme extract.

The POX activity was measured in 50 mM phosphate buffer (pH 5.5) containing 1 mM guaiacol and 0.5 mM H_2O_2 by monitoring the increase in absorbance at 470 nm (Janda *et al*., 2003). One unit (U) of POX activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 min^{-1}.

The CAT activity was measured in 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2 by monitoring the decrease in absorbance at 240 nm. One unit (U) of CAT activity was defined as the amount of enzyme that used 1 μmol H_2O_2 min^{-1} (Gong *et al*., 2001).

The SOD activity was estimated according to the method of Agarwal and Pandey (2004). One unit (U) of enzyme activity was taken as that amount of enzyme, which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme.

Lipid peroxidation

For measuring the lipid peroxidation in seeds, the thiobarbituric acid (TBA) test, which deter-

mines malondialdehyde (MDA) as an endproduct of lipid peroxidation, was used. Lipid peroxidation was assessed as described by Velikova *et al.* (2000) measuring the MDA content. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

Determination of H_2O_2 content

Hydrogen peroxide levels were measured by monitoring the absorbance at 410 nm of the titanium peroxide complex according to He *et al.* (2005). Absorbance values were calibrated to a standard curve generated with known concentrations of H_2O_2.

Statistical analysis

Each experiment was repeated at least three times with two replicates. Statistical analysis was performed using one way analysis of variance (ANOVA). $P \leq 0.05$ was considered as significant.

Results and Discussion

As seen from the results summarized in Table I, at all concentrations, progesterone and β-estradiol augmented the germination speed of chickpea seeds compared to the control. The maximum seed germination was recorded as 78 and 100% for 10^{-6} M progesterone-treated seeds and 76 and 100% for 10^{-9} M β-estradiol-treated seeds at the

Table I. Effects of progesterone and β-estradiol on morphologic and biochemical parameters of germinating chickpea seeds.

Parameter	Time (day)	Hormone concentration [M]					Range of standard deviation
		Control	Progesterone		β-Estradiol		
		0	10^{-9}	10^{-15}	10^{-9}	10^{-15}	
Seed germination (%)	1st	62	75	63	76	66	2.80–2.88
	3rd	85	96	88	100	90	0.25–2.89
	5th	100	100	100	100	100	0–0
Root length [cm]	1st	0.41	0.53	0.435	0.63	0.625	0.01–0.04
	3rd	3.36	4.25	3.28	3.88	3.55	0.04–0.07
	5th	5.70	6.41	6.02	6.88	5.88	0.01–0.06
Shoot length [cm]	3rd	0.74	0.94	0.77	1.17	1.02	0.01–0.02
	5th	2.52	2.84	2.56	3.19	2.72	0.01–0.04
α-Amylase activity [U g^{-1}]	1st	157	180	166	237	227	1.52–3.51
	3rd	166	236	203	265	261	1.52–4.04
	5th	200	263	235	269	258	1.15–4.16
SOD activity [U g^{-1}]	1st	13.6	41	21	29.3	16.4	0.57–1.54
	3rd	29.4	48.2	24	53.8	34	1.12–1.52
	5th	38.1	57.4	41.4	60.5	46.6	0.78–1.96
H_2O_2 content [μmol g^{-1} FW]	1st	94	122	98	119	99	0.57–2.08
	3rd	177	277	211	256	209	1.73–4.61
	5th	116	142	123	233	149	0.57–4.35
POX activity [U g^{-1}]	1st	325	328	325	331	325	0.57–1.15
	3rd	3356	4800	4130	4430	4360	21–134
	5th	10900	15580	12300	17580	14410	80–196
CAT activity [U g^{-1}]	1st	145	228	168	236	150	1.01–4.35
	3rd	1023	1170	1065	1213	1078	2.08–11.52
	5th	1233	1300	1240	1365	1265	2.64–6.51
MDA content [nmol ml^{-1} FW]	1st	0.645	0.465	0.59	0.48	0.59	0.014–0.021
	3rd	0.97	0.8	0.93	0.62	0.88	0.015–0.020
	5th	2.48	2.32	2.39	2.14	2.38	0.011–0.025

All values are means of at least three determinations with 2 replicates.

end of the 1st and 3rd day, respectively; 62 and 85% germination in control seeds was observed in the same time periods. All groups (control and steroid-treated seeds) showed 100% germination at the end of the 5th day. These results were in agreement with those reported in previous studies, but the obtained optimal concentrations were lower than in the prior studies. Some researchers informed that pea embryos were stimulated by estrone (Helmkamp and Bonner, 1952; Kögl and Haagen-Smit, 1936). They claimed that best optimal concentration range was 10^{-6}–10^{-8} M. Besides, Martínez-Honduvilla *et al.* (1976) reported that the mammalian steroids (estrone, β-estradiol and testosterone) increased the germination degree and growth rate compared to the control.

Similarly, at all tested concentrations, although, both progesterone and β-estradiol meaningfully augmented the root and shoot lengths of chickpea seeds compared to the control, 10^{-6} M progesterone and 10^{-12} M β-estradiol supplied the best ameliorative effects on these parameters. These results were similar to previous studies, which showed that estrone (1 mg l^{-1}) stimulated the growth of an isolated pea embryo *in vitro* and accelerated the meristem activity in the roots of *Melandrium dioecium*, *Rumex acetosa*, and *Anthoxanthum aristatum*. In germinating wheat seeds, root and shoot lengths increased at low concentrations (10^{-8} and 10^{-6} M) more than at higher concentrations (10^{-4} M) of the steroids (Dogra and Kaur, 1994). These results suggested that progesterone and β-estradiol might stimulate the root and shoot growth depending on the increment of germination velocity. It can be also said that these steroids (especially β-estradiol) may be used to accelerate the seed germination and seedling growth.

Seeds gather the needed storage materials in cotyledons or endosperm (Bewley and Black, 1994). Embryos provide energy that is needed for germination from the degradation of storage forms such as carbohydrates. The beginning of germination activates the production of hydrolyzed enzymes, especially α-amylase. This enzyme plays a major role in degradation of reserve carbohydrates to soluble sugars, which are used for providing the needed energy in germination (Perata *et al.*, 1997; Vartapetian and Jackson, 1997). Hence, α-amylase is critical in seed germination. The α-amylase activity was augmented by all concentrations of both progesterone and β-estradiol. The highest activity values were determined in the 10^{-6} M progesterone-treated seeds as 203, 259 and 272 U g^{-1} and in the 10^{-12} M β-estradiol-treated seeds as 270, 275 and 281 U g^{-1}, respectively, at the end of the 1st, 3rd and 5th day. This finding is in accordance with the results of preceding investigators, who showed that these steroids stimulated the α-amylase activity in germinating seeds. But, the optimal hormone concentrations obtained from the present study were lower than the optimal values 10^{-6}–10^{-8} M by these investigators (Dogra and Kaur, 1994; Dogra and Thukral, 1991). With respect to these results, an increase in germination velocity may attribute to an increment in α-amylase activity.

On the other hand, it has been known well that in plants reactive oxygen species (ROS) are permanently produced in cells even under optimal conditions (Atıcı and Nalbantoglu, 2003; Kang *et al.*, 2003). The tolerance of plants to oxidative stress conditions may be attributed to the increase in the activity of one or more antioxidant enzymes preventing cell and tissue damage. To determine the effects of progesterone and β-estradiol on the antioxidant enzymes in germinating chickpea seeds, the activities of three antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), were studied. SOD transforms superoxide to H_2O_2 by acting as the first line of defence against ROS. Since H_2O_2 is a substance toxic to living cells, POX and CAT metabolize H_2O_2 to H_2O (Willekens *et al.*, 1997). In the present study, it was determined that the SOD, POX, and CAT activities were significantly increased by all concentrations of progesterone and β-estradiol compared to the control. The maximum SOD activities were 47, 53, 62 U g^{-1} for 10^{-6} M progesterone and 29, 54, 61 U g^{-1} for 10^{-9} M β-estradiol at the end of the 1st, 3rd, and 5th day, respectively. The highest CAT activities were recorded as 230, 1200, and 1336 U g^{-1} for 10^{-6} M progesterone and 236, 1213, and 1365 U g^{-1} for 10^{-9} M β-estradiol at the end of the 1st, 3rd, and 5th day, respectively. The maximum POX activities were determined as 328, 4800, and 15580 U g^{-1} for 10^{-6} M progesterone and 333, 4640, and 17230 U g^{-1} for 10^{-12} M β-estradiol at the end of the 1st, 3rd, and 5th day, respectively. On the other hand, the H_2O_2 content was also augmented by these steroids. The maximum values for the H_2O_2 level were achieved with 10^{-6} M progesterone and 10^{-9} M β-estradiol that resulted in the maximum SOD activities. The increment of

the H_2O_2 level may be attributed to the increment of SOD activity.

Oxidative damage, caused by ROS, interrupts the lipid metabolism (Hernandez and Almansa, 2002; Misra and Gupta, 2006). The final product of lipid peroxidation, malondialdehyde (MDA), is poisonous to plant cells. The MDA amount could be expressed as degree of lipid peroxidation. In the present study, it was found that the MDA contents of chickpea seeds at the 1st, 3rd, and 5th day of germination were significantly decreased by progesterone and β-estradiol. MDA contents were lowest at the concentrations 10^{-6} M progesterone and 10^{-9} M β-estradiol, where antioxidant enzyme activities were the highest. The decrease of MDA content may be attributed to the increasing activities of antioxidant enzymes (SOD, CAT and POX) detoxifying ROS. Considering these results, it is possible to say that progesterone and β-estradiol may prevent possible damages, caused by ROS, by increasing the SOD, POX and CAT activities.

It was determined that at all tested concentrations of both progesterone and β-estradiol accelerated the seed germination and post-germination growth in chickpea by causing important biochemical changes. These results are basically in agreement with previous studies. However, the optimal concentrations of steroids obtained in the present study were lower than those obtained in previous studies. This finding is very important due to the fact that, when the steroids are applied at lower concentrations, their possible negative side effects may decrease. Therefore, steroids may be used at low concentrations in agriculture. Because of positive effects on the germination velocity of the steroids, they may be used to accelerate the seed germination in some plant species which have germination difficulty. On the other hand, the steroids may be used to supply resistance to plants under various stress conditions since they have a stimulating effect on the antioxidant enzyme activity. The optimal concentrations of steroids were determined as 10^{-6}–10^{-8} M in previous studies, while the optimal concentrations in the present study were 10^{-6} M progesterone and 10^{-9} and 10^{-12} M β-estradiol. From the results mentioned above, we believe that the usage of these steroids in agriculture can be very advantageous owing to roles at both the acceleration of germination and reduction of oxidative damage by increasing enzyme activity. However, further studies are needed to prove the usability of steroids in agriculture.

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Agarwal S. and Pandey V. (2004), Antioxidant enzyme responses to NaCl stress in *Cassia angustifoli.* Biol. Plant. **48**, 555–560.

Aisien A.-O., Palmer G.-H., and Stark J.-R. (1983), The development of enzymes during germination and seedling growth in Nigerian sorghum. Staerke **35**, 316–320.

Atıcı O. and Nalbantoglu B. (2003), Antifreeze proteins in higher plants. Phytochemistry **64**, 1187–1196.

Atici O., Agar G., and Battal P. (2005), Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress. Biol. Plant. **49**, 215–222.

Barrington E.-J.-W. (1979), In: Hormones and Evolution (Barrington E.-J.-W., ed.). Academic Press, London, pp. 7–21.

Bewley J.-D. and Black M. (1994), Seeds: Physiology of Development and Germination, 2nd ed. Plenum Press, New York, London, pp. 1–31.

Bhattacharya B. and Gupta K. (1981), Steroid hormone effects on growth and apical dominance of sunflower. Phytochemistry **20**, 989–991.

de Morais G.-A. and Takaki M. (1998), Determination of amylase activity in cotyledons of *Phaseolus vulgaris* L. cv. *carioca*. Braz. Arch. Biol. Technol. **41**, 17–25.

Dogra R. and Thukral A. (1991), Effect of steroid and plant hormones on some germination aspects of *Triticum aestivum* L. In: New Trends in Plant Physiology (Dhir K. K., Dua I. S., and Chark K. S., eds.). Today and Tomorrow's Printers and Publishers, New Delhi, pp. 65–70.

Dogra R. and Kaur A. (1994), Effect of steroids on some growth and biochemical parameters of *Triticum aestivum* L. during germination. Crop. Res. **8**, 611–620.

Dohrn M., Faure W., Poll H., and Blotevogel W. (1926), Tokokinine, Stoffe mit sexualhormonartiger Wirkung aus Pflanzenzellen. Med. Klin. **22**, 1417–1419.

Geuns J.-M.-C. (1978), Steroid hormones and plant growth and development. Phytochemistry **17**, 1–14.

Geuns J.-M.-C. (1982), Plant steroid hormones – what are they and what do they do? Trends Biochem. Sci. **7**, 7–9.

Gong Y., Toivonen P.-M.-A., Lau O.-L., and Wiersma P.-A. (2001), Antioxidant system level in 'Braeburn' apple is related to its browning disorder. Bot. Bull. Acad. Sin. **42**, 259–264.

Hayat S., Ahmad A., Hussain A., and Mobin M. (2001), Growth of wheat seedlings raised from the grains treated with 28-homobrassinolide. Acta Physiol. Plant. **23**, 27–30.

He Y., Liu Y., Cao W., Huai M., Xu B., and Huang B. (2005), Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in *Kentucky bluegrass*. Crop Sci. **45**, 988–995.

Heftman E., Ko S.-T., and Bennett R.-D. (1965), Identification of estrone in date seeds by thin-layer chromatography. Naturwissenschaften **52**, 431–432.

Helmkamp G. and Bonner J. (1952), Some relationships of sterols to plant growth. Plant Physiol. **28**, 428–436.

Hernandez J.-A. and Almansa M.-S. (2002), Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant. **115**, 251–257.

Iglesias R.-G. and Babiano M.-J. (1997), Endogenous abscisic acid during the germination of chickpea seeds. Physiol. Plant. **100**, 500–504.

Janda T., Szalai G., Rios-Gonzales K., Veisa O., and Paldi E. (2003), Comparative study of frost tolerance and antioxidant activity in cereals. Plant Sci. **164**, 301–306.

Janeczko A. and Skoczowski A. (2005), Mammalian sex hormones in plants. Folia Histochem. Cytochem. **43**, 71–79.

Jones J.-L. and Roddick J.-G. (1988), Steroidal estrogens and androgens in relation to reproductive development in higher plants. J. Plant Physiol. **133**, 156–164.

Juliano B.-O. and Varner J.-E. (1969), Enzymic degradation of starch granules in the cotyledons of germinating peas. Plant Physiol. **44**, 886–892.

Kang G., Wang C., Sun G., and Wang Z. (2003), Salicylic acid changes activities of H_2O_2-metabolizing enzymes and increases the chilling tolerance of banana seedlings. Environ. Exp. Bot. **50**, 9–15.

Kato-Noguchi H. and Macias F.-M. (2005), Effects of 6-methoxy-2-benzoxazolinone on the germination and α-amylase activity in lettuce seeds. J. Plant Physiol. **162**, 1304–1307.

Kliewer S.-A., Moore J.-T., Wade L., Staudinger J.-L., Watson M.-A., Jones S.-A., McKee D.-D., Oliver B.-B., Willson T.-M., Zetterstrom R.-H., Perlmann T., and Lehmann M. (1998), An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell **92**, 73–82.

Kögl F. and Haagen-Smit A.-J. (1936), Biotin und Aneurin als Phytohormone. Z. Physiol. Chem. **243**, 209–227.

Kopcewicz J. (1970), Influence of estrogens on the flower formation in *Cichorium intybus* L. Naturwissenschaften **57**, 136–137.

Marero L.-M., Payumo E.-M., Librando E.-C., Lainez W.-N., Gopez M.-D., and Homma S. (1988), Technology of weaning food formulations prepared from germinated cereals and legumes. J. Food Sci. **9**, 17–33.

Martínez-Honduvilla C.-J., Gimenez-Solves A., and Santos-Ruiz A. (1976), Biochemical changes in *Pinus pinea* seeds. III. The effect of growth substances and steroidal hormones on nucleic acids. Rev. Esp. Fisiol. **32**, 169–185.

Misra N. and Dwivedi U.-N. (2004), Genotypic difference in salinity tolerance of green gram cultivars. Plant Sci. **166**, 1135–1142.

Misra N. and Gupta A.-K. (2006), Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings. J. Plant Physiol. **163**, 11–18.

Mordue W. and Stone J.-V. (1979), In: Hormones and Evolution (Barrington E. J. W., ed.). Academic Press, London, pp. 215–273.

Paleg L.-G. (1960), Physiological effects of gibberellic acid I. On carbohydrate metabolism and amylase activity of barley endosperm. Plant Physiol. **35**, 293–299.

Perata P., Guglielminetti L., and Alpi A. (1997), Mobilization of endosperm reserves in cereal seeds under anoxia. Ann. Bot. **79**, 49–56.

Sandor T. and Mehdi A.-Z. (1979), In: Hormones and Evolution (Barrington E. J. W., ed.). Academic Press, London, pp. l–72.

Shore L.-S., Kapulnik Y., Ben-Dor B., Fridman Y., Wininger S., and Shemesh M. (1992), Effects of estrone and 17β-estradiol on vegetative growth of *Medicago sativa*. Physiol. Plant. **84**, 217–222.

Simersky R., Novak O., Morris D.-A., Pouzar V., and Strnad M. (2009), Identification and quantification of several mammalian steroid hormones in plants by UPLC-MS/MS. J. Plant Growth Regul. **28**, 125–136.

Simons R.-G. and Grinwich D.-L. (1989), Immunoreactive detection of four mammalian steroids in plants. Can. J. Bot. **67**, 288–296.

Skarzynski B. (1933), An oestrogenic substance from plant material. Nature **131**, 766.

Vartapetian B.-B. and Jackson M.-B. (1997), Plant adaptations to anaerobic stress. Ann. Bot. **79**, 3–20.

Velikova V., Yordanov I., and Edreva A. (2000), Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. Plant Sci. **151**, 59–66.

Willekens H., Chamnongpol S., Davey M., Schraudner M., Langebartels C., and Van Montagu M. (1997), Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. EMBO J. **16**, 4806–4818.

Ylstra B., Touraev A., Brinkmann A.-O., Heberle-Bors E., and Tunen A. (1995), Steroid hormones stimulate germination and tube growth of *in vitro* matured tobacco pollen. Plant Physiol. **107**, 639–643.