Annex 1 to advice 07-2013 (dossier 2012/07): Mammalian steroids in plants

The presence of progesterone in plants was first reported in 1960's by Gawienowski and Gibbs. They estimated the amount of progesterone at 500 ng/g in apple seed (*Mallus Sp*.). Extensive studies of the occurrence of mammalian steroids in plants (128 species from 50 families) were carried out using radioimmunoassay (RIA) in 1989 by Simons and Grinwich. Androsterone and progesterone were found in more than 80% of the investigated species, androgens (testosterone and dihydrotestosterone) in 70% of species and estrogens (estrone and 17β-estradiol) in 50% of species. The level of progesterone determined in some plant tissues exceeded 2600 ng/g dry weight (d.w.) (Iino *et al.,* 2007). The content of steroids may significantly change during plant development and is dependent on the species, cultivar and plant organ (table 1). For example, the concentration of progesterone in the leaves of *Prunus virgniana L.* was found to be 13 ng/g d.w. In the flower buds, the concentration increased to 58 ng/g, but then in the flowers it again decreased to 7 ng/g (Janeczko, 2012). Zhang *et al.* (1991), also using the RIA method, estimated the content of total estrogens and 17β-estradiol in pollen and in the style of *Ginkgo biloba* L., *Zea mays* L. and *Brassica campestris* L. 17β-estradiol was present in the pollen of these plants in the range of 0.008-0.035 ng/g fresh weight (f.w.). In the style of *Lilium davidi* Duch*.,* 17β-estradiol was found in the concentration of 0.024-0.040 ng/g f.w. Moreover, the mentioned authors showed changes of total estrogen and 17β-estradiol concentrations during flower development. Zhong-han *et al*. (1994) proved the presence of testosterone in the pollen of *Pinus bungeana* Zucc. ex Endl. (11 ng/g d.w.), *G. biloba* (87 ng/g d.w.) and *Pinus tabulaeformis* Carr. (27 ng/g d.w.) using the ELISA method. Testosterone was not detected in the pollen of *Juglans regia* L. and *B. campestris*. According to the authors, testosterone was also present in the pistil of *L. davidi*. (Janeczko and Skoczowski, 2005).

Hartmann *et al*. (1998) measured the concentration of steroid hormones in food. Progesterone was found in the more obvious products of animal origin, such as meat and eggs, but also in steamed potatoes (5.07 ng/g), wheat meal (up to 2.86 ng/g), parboiled rice (0.38 ng/g), natural olive oil (0.08 ng/g) and refined corn oil (0.31 ng/g).

Concentration of progesterone measured by Iino *et al.* (2007) is presented in table 2. The richest source was shoots of rice although reproductive tissues such as inflorescences, seeds and ears generally contained higher levels. This work demonstrated that the amounts of progesterone in plant tissues were generally in the order of ca. 1 µg or less per kg f. w. of tissue (Iino *et al*., 2007). Simerský *et al*. (2009) detected the presence of progesterone in *nicotiana tabacum* L. (55.46 pmol/g fw.), *digitalis purura* (58.92 pmol/g f.w.) and *Inulla helenium* L. (2.10 pmol/g f. w.). Exctract of D*. purpurea* also contained 16-dehydroprogesterone (29 pmol/g f.w.) and 17 α-hydroxyprogesterone (173 pmol/g f.w.). Extract of *N. tabacum* and *I. helenium* also contained androstendione in concentrations of 7.7 pmol/g f.w. and 11 pmol/g f.w., respectively. Pauli *et al*. (2010) found progesterone in *juglans regia,* a vascular plant. Progesterone is naturally present in wheat seedlings in its conjugate form (as glycosides). The estimated amount of progesterone in the hydrolyzed extract was four times higher (2.29 pmol/g f. w.) than in untreated extracts (0.55 pmol)(Janeczko, 2012).

Table 1: Occurrence of mammalian sex hormones and related mammalian steroids in selected plants, estimated by radioimmunoassay in 1989 by Simons and Grinwich modified by Janeczko and Skoczowski (2005).

Species and organs	Date (collected)	Androgens (ng·g d.w. $^{-1}$)	Androstenone (ng·g d.w. $^{-1}$)	Estrogens (ng·g d.w. $^{-1}$)	Progesterone (ng·g d.w. $^{-1}$)
Triticum aestivum L. cv. Glenlea (leaf tissue) cv. Benito (leaf tissue)	7 July	 0 0	 220 140	 0 0	 0 0
Hordeum vulgare L. (leaf tissue)	14 May	41	0	78	31
Monstera deliciosa Liebm. (leaf tissue)	27 April	140	8400	420	84
Zea mays L. (leaf tissue)	12 May	170	0	86	280
Prunus virginiana L. (leaf tissue)	20 May	140	1300	34	13
Brassica campestris L. cv. Torch (leaf tissue)	7 July	0	250	0	6
Crassula arborescens Willd. (leaf tissue)	27 April	3200	1300	320	130
Daucus carota L. (stem tissue)	5 June	0	310	0	15
Urtica dioica L. (shoot tissue)	30 April	99	0	500	79
Thlaspi arvense L. (shoot tissue)	29 April	95	190	240	76
Acer negundo L. (female influorescence tissue, male influorescence tissue)	30 April	 49 82	 0 41	 98 82	 10 8
Syringa vulgaris L. (influorescence tissue - early bud, influorescence tissue - full bloom)	 24 May 24 June	 44 0	 180 270	 44 0	 18 0
Bromus inermis Leyss. (mature seeds)	-	44	0	0	18
Brassica napus L. cv. Oro (mature seeds)	-	11	0	0	9

Table 2 :Concentration of progesterone measured by Iino *et al.* (2007)

Plant species	**Tissue analyzed**	**Progesterone content (ng/kg f.w.)**
Arabidopsis thalinan L.	shoots and	160
Arabidopsis thalinan L.	inflorescences	400
Pea (*Pisum sativum)*	shoot	190
Pea (*Pisum sativum)*	root	260
Pea (*Pisum sativum)*	mature seed	410
Adzuki bean (*Phaseolus angularis*)	etiolated seedling	48
Mung bean (*Vigna radiata*)	etiolated seedling	21
Tomato (*Lycopersicon esculentum*)	leaf	25
Tomato (*Lycopersicon esculentum*)	immature green fruit	280
Tomato (*Lycopersicon esculentum*)	mature red fruit	6
Potato (*Solanum tuberosum*)	tuber	24
Apple (*Malus domestica*)	flesh	150
Apple (*Malus domestica*)	seed	430
Rice (*Oryza sativa)*	shoot	1540
Rice (*Oryza sativa)*	ear	440
Onion (*Allium cepa*)	bulb	68

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