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THE EFFECT OF MAMMALIAN SEX HORMONES ON *IN VITRO* PROPERTIES OF SAINFOIN (*ONOBRYCHIS SATIVA* L.)

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ABSTRACT

This study was prepared to determine reactions of mammalian sexual hormones (MSH) (progesterone, 17β-estradiol, estrone and testosterone) on sainfoin plants (*Onobrychis sativa* L.) grown in *in vitro* conditions, which was applied for 13 different methods performed in two stages. In the first stage, the seeds were cultured in hormone-free medium. In the second stage, the explants (cotyledon, root and hypocotyl) obtained from the plantlets were transferred to the medium including different hormone, dose and hormone-dose combinations. For the effects of MSH, the highest shoot (16 shoot/petri) were obtained from hypocotyl explants with the doses 10^{-6} mM of progesterone and, 10^{-4} and 10^{-6}'mM of estrone; the highest rate of callus formation (84,4 %) with the dose 10^{-4} mM of estrone; the highest vigor rate (73,4%) with the doses 10^{-4} and 10^{-5} mM of progesterone from root explants were obtained. For rooting, the most suitable hormone was testosterone, the most suitable explant was hypocotyl and it is observed that number of root increased depending on treatment day. This study is the primary in terms of determination of the effects of MSH on tissue culture of sainfoin (*Onobrychis sativa* L).

KEYWORDS:
Onobrychis sativa L., mammalian sex hormones, tissue culture, sainfoin, plant hormones

INTRODUCTION

In today's world where transformation to nature has become a slogan, plants have vital importance. Because of the plant species which have more than 350.000 and the vital roles that unlike the other living groups, plants are considered as the key of the life on the earth. Besides, plants are used in cosmetics and pharmaceutical industry, they provide habitat for vast array of the living creatures, they provide organic substance for soil, environmental planning, play an important role in preventing natural disasters, and processed as raw material in industry [1, 2, 3]. Sainfoin has significant amount of adaptive skills and resistant to cold and drought in perennial forage crop. Sainfoin that has wide range of utilization area brings forth high-end harvest. That sainfoin alone or mixed with poaceae, is used in haymaking, pasture improvement, soil improvement, honey production, crop rotation [4]. Producing new crop varieties which combines desirable characteristics by plant breeding is tough and time-taking process. It is possible to encounter some problems such as incompatibility, genetic dependency and time-taking process of the selection and stabilization of desirable characteristics. However, by the help of the widespreading techniques of plant genetic engineering, these problems can be easily overcome. Without manipulating the original plant's desired characteristics one or several gene(s) that are isolated from different organisms can be transferred to other plants [5]. Mamal steroid sex hormones (MSSH) (progesterone, 17β-estradiol, estrone and testosterone) are found naturally in plants. But their substances vary by species, plant organs and their stages of development [6]. Mamal steroid sex hormones was detected in 1926 for the first time. Since then, many researches have been conducted on the formation of mamal steroid sex hormones and physiological activities of mamal steroid sex hormones, but mechanisms of action and metabolism of these hormones still can't be totally illuminated. Because they have been detected in reproductive organs, it is thought that they have influence on inflorescence and reproduction and they aren't regarded as plant growth regulators [7, 8, 9]. From past to present, some researches on sainfoin have been conducted and various factors have been examined. However, the researches related to mamal steroid sex hormones are very few when compared to all the researches. In this study that was carried by using seeds of sainfoin (Lütfibey), the effects of four different concentration of the certain mamal steroid sex hormones namely progesterone, 17β-estradiol and testosterone, estrone (0, 10^{-4} mM, 10^{-5} mM ve 10^{-6} mM) on *in vitro* properties were examined.

TABLE 1
Chemicals and quantities used in the experiment

Chemicals	Applications												
	1	2	3	4	5	6	7	8	9	10	11	12	13
MS Salts[1] (g/l)	4,43	4,43	4,43	4,43	4,43	4,43	4,43	4,43	4,43	4,43	4,43	4,43	4,43
MS Vitamins (1000x)[2] (ml/l)	1	1	1	1	1	1	1	1	1	1	1	1	1
Sucrose[1](g/l)	20	20	20	20	20	20	20	20	20	20	20	20	20
17β –estradiol[2] (mM)	0	10^{-4}	10^{-5}	10^{-6}									
Testosterone[2] (mM)	-				10^{-4}	10^{-5}	10^{-6}						
Progesterone[2] (mM)	-							10^{-4}	10^{-5}	10^{-6}			
Estrone[2] (mM)	-										10^{-4}	10^{-5}	10^{-6}
MES[1] (g/l)	1,95	1,95	1,95	1,95	1,95	1,95	1,95	1,95	1,95	1,95	1,95	1,95	1,95
Ascorbic Acid (50 mg/l)[2] (ml)	2	2	2	2	2	2	2	2	2	2	2	2	2
Agar[1] (g/l)	7	7	7	7	7	7	7	7	7	7	7	7	7

[1]: Heat- labile compound, [2]: Thermolabile compound

MATERIALS AND METHODS

In this study, the seeds that belong to Lütfibey sainfoin (*Onobrychis sativa* L.) provided by East Anatolian Agricultural Research Institute were used. After the seeds' testa dehulled, seeds were rinsed with distilled water and then they were surface sterilized with 70% ethanol for 15 min and with 1% sodium hypochlorite (20% commercial bleach) for 30 min and then rinsed with sterile water. And then, the seeds were aseptically germinated in Murashige and Skoog (MS) medium and vitamins in petri dishes. They were kept at 25°C under a 16h light, 8h dark period. Cotyledon, root, hypocotyl of the plantlets that groved were used as explant. In the first stage, seeds were germinated on standart MS medium and explants were taken. In the second stage explants (cotyledon, root, hypocotyl) were cultured on the medium that including four different concentration of the certain mamal steroid sex hormones namely progesterone, 17β-estradiol and testosterone, estrone (0, 10^{-4} mM, 10^{-5} mM and 10^{-6} mM). 30 days after this procedure explants were transferred to hormone-free medium. At the end of 30. and 60. days, explants were transferred to new mediums according to viability of explants, observations were recorded at the end of the 30, 60 and 90. days. As a medium MS salt and vitamins Murashige and Skoog [10], as carbohydrate source 20 gr l^{-1} sucrose, 100 mg l^{-1} ascorbic acid, 7 g l^{-1} agar, 1.95 gr l^{-1} MES were used. The pH of this medium was adjusted to 5,8. Explants were cultured at 25°C under 16h light, 8h dark. Fluorescent tubes that were 1500 lux were used as a source of light. Each petri regarded as repetition and 16 explants were put in per petri dishes. This study was conducted according to completely randomized design with 4 replications. The experiment was designed and analyzed according to 4x4 factorial experimental designs consisting of 4 different hormone types and 4 different hormone doses that belong to these hormones. The results obtained was determined by analyses of variance (ANOVA) by SAS GLM (SAS Inst., Cary, NC) computer program and it was estimated at 0.05 probability level according to DUNCAN's multiple range test.

RESULTS AND DISCUSSION

Effects of MSH on the capacity of shoot generation from cotyledon. When taking the effects of hormone types on shoot generation according to mean of applied dose into consideration, it was seen that 17β-estradiol with averagely 0,8 shoot was placed on the top. The number of shoot was 0,3 in control (Table 2). On the other hand the most shoots were obtained from 10^{-5} mM dose with 0,6 shoots when the results were examined according mean of hormone doses. All the doses gave better results when compared to the control (Table 2). When hormone types were examined separately according to their doses, it was seen that the most shoot generation was observed in these doses: progesterone (10^{-4} mM) and 17β-estradiol (10^{-6} mM) with 1,3 shoots (Table 2).

Effects of MSH on the viability rate of cotyledons. In regard to cotyledons' viability rates hormone X dose interaction was very important. ($p<0,01$). When considering mean values of hormone types, estrone had the highest viability rate (19,1%). The rate of viability determined as 12,5% in control. On the other hand, when taking mean of hormone doses into consideration the highest viability rate was found in 10^{-4} mM dose (19,1%). As it is seen from these datum, the rate of viability of the mean of all hormone doses was higher than the rate of control. When hormone types were examined separately according to applied doses, testosterone's 10^{-6} mM dose showed the highest viability rate (34,4 %) (Table 2).

Effects of MSH on the shoot generation capacity of hypocotyls. Effects of hormone types and doses were very important, according to results of variance analysis that belongs to the number of shoot-forming explants. In the same way, effect of interaction hormone X dose became very important (Table 3). When 12 combinations were examined separately according to hormone types and doses, in terms of the shoot-forming explants, progesterone's 10^{-6} mM dose with 16,0 shoots and estrone's 10^{-4} mM and 10^{-6} mM doses were placed on the first (Table 3).

When the mean value of hormone types given in Table 3 analyzed, it was seen hypocotyl used as explant, estrone with 15,9 shoots took the first place. Besides, according to the means of the hormone doses, the number of shoot-forming explants in the control (16,0 shoots) was higher than the means of all the hormone doses and 10^{-6} mM dose with 13,1 shoots in the first place (Table 3).

Effects of MSH on the callus formation of hypocotyl. Effect of hormone X dose interaction on the callus formation was very important ($p<0,01$). When the mean values of hormone types is taken into account, the highest rate of callus formation obtained from progesterone (3,5 %). But, it was seen that when it was analyzed according to the mean of hormone doses the highest rate callus formation obtained from (3,5 %) 10^{-5} mM dose. It was also determined that callus wasn't generated in the control. When hormone types were examined separately according to applied doses, the highest callus formation rate was obtained from progesterone's 10^{-5} mM dose (14,1 %) and it was followed by testosterone's 10^{-4} mM dose (4,7 %) (Table 3).

TABLE 2
Number of shoot-forming from cotyledon explants at the end of 90th day according to different hormone types and doses, viability rate at the end of 90th day

	Hormone	Doses (mM)				
		0	10^{-4}	10^{-5}	10^{-6}	Mean
The number of shoot forming explant at the end of 90th day (shoot /petri)	Progesterone	0,3	1,3	0,5	0,3	0,6
	Estrone	0,3	0,0	0,8	0,3	0,3
	17β-estradiol	0,3	0,5	1,0	1,3	0,8
	Testosterone	0,3	0,3	0,3	0,0	0,2
	Mean	0,3	0,5	0,6	0,4	-
The viability rate at the end of 90th day (%)	Progesterone	$12,5^{b}$	$28,1^{a}$	$14,1^{b}$	$0,0^{c}$	13,7
	Estrone	$12,5^{a}$	$18,8^{a}$	$25,0^{a}$	$20,3^{a}$	19,1
	17β-estradiol	$12,5^{a}$	$3,1^{a}$	$12,5^{a}$	$10,9^{a}$	9,8
	Testosterone	$12,5^{bc}$	$26,6^{ab}$	$1,6^{c}$	$34,4^{a}$	18,8
	Mean	12,5	19,1	13,3	16,4	-

1) The differences between the means shown on the same line with the same lower-case letter are insignificant.

TABLE 3
Number of shoot-forming explant from hypocotyl at the end of 30th day according to different hormone types and doses, callus formation rate at the end of the 30th day, number of root-forming explants at the end of 30th and 60th day

	Hormone	Doses (mM)				
		0	10^{-4}	10^{-5}	10^{-6}	Mean
Number of shoot forming explants at the end of 30th day (shoot /petri)	Progesterone	$16,0^{a}$	$15,3^{a}$	$13,5^{b}$	$16,0^{a}$	$15,2^{A}$
	Estrone	$16,0^{a}$	$16,0^{a}$	$15,5^{b}$	$16,0^{a}$	$15,9^{A}$
	17β-estradiol	$16,0^{a}$	$12,8^{b}$	$12,0^{b}$	$12,5^{b}$	$13,3^{B}$
	Testosterone	$16,0^{a}$	$3,5^{c}$	$5,0^{bc}$	$7,8^{b}$	$8,1^{C}$
	Mean	$16,0^{a}$	$11,9^{bc}$	$11,5^{c}$	$13,1^{b}$	-
Callus formation rate at the 30th day (%)	Progesterone	$0,0^{b}$	$0,0^{b}$	$14,1^{a}$	$0,0^{b}$	3,5
	Estrone	0,0	0,0	0,0	0,0	0,0
	17β-estradiol	0,0	0,0	0,0	0,0	0,0
	Testosterone	$0,0^{a}$	$4,7^{a}$	$0,0^{a}$	$0,0^{a}$	1,2
	Mean	0,0	1,2	3,5	0,0	-
Number of root-forming explant at the end of 30th and 60th. day (root /petri)	Progesterone	$4,8^{a}$	$3,5^{a}$	$1,0^{c}$	$2,0^{b}$	$2,8^{D}$
	Estrone	$4,8^{a}$	$1,5^{c}$	$2,4^{b}$	$7,4^{a}$	$4,0^{C}$
	17β-estradiol	$4,8^{b}$	$6,3^{a}$	$6,8^{a}$	$6,3^{a}$	$6,0^{B}$
	Testosterone	$4,8^{c}$	$8,3^{b}$	$9,9^{ab}$	$11,0^{a}$	$8,5^{A}$
	Mean	$4,8^{b}$	$4,9^{b}$	$5,0^{b}$	$6,7^{a}$	-

1) The differences between the means shown at the same column with the same capital letter and at the same line with the same lower-case letter are insignificant

TABLE 4
Number of root-forming explants at the end of 30th and 60th day from hypocotyl according to different hormone types and doses

		Number of treatment days		
		30	**60**	**Mean**
Hormone	**Progesterone**	$0,0^{d}$	$5,6^{b}$	$2,8^{D}$
	Estrone	$1,0^{c}$	$7,0^{b}$	$4,0^{C}$
	17β-estradiol	$2,1^{b}$	$9,9^{a}$	$6,0^{B}$
	Testosterone	$6,7^{a}$	$10,3^{a}$	$8,5^{A}$
	Mean	2,5	8,2	-
Doses (mM)	**0**	$0,0^{c}$	$9,5^{a}$	$4,8^{B}$
	10^{-4}	$2,6^{b}$	$7,1^{b}$	$4,9^{B}$
	10^{-5}	$3,3^{ab}$	$6,8^{b}$	$5,1^{B}$
	10^{-6}	$3,9^{a}$	$9,4^{a}$	6,7
	Mean	2,5	8,2	-

1) The difference between the means shown at the same column with the same capital letter and at the same column with the same lower-case letter are insignificant

TABLE 5
Number of explants forming roots according to different hormone types and doses, callus formation rate at the end of the 30th day, viability rate at the end of 90th day, number of shoots rooted at the end of 90th day, number of shoots at the end of 90th day.

	Hormone	Doses (mM)				
		0	**10^{-4}**	**10^{-5}**	**10^{-6}**	**Mean**
Number of shoot forming explant (shoot /petri)	**Progesterone**	5,8	5,5	7,3	9,3	$7,0^{A}$
	Estrone	5,8	0,8	4,3	4,8	$3,9^{B}$
	17β-estradiol	5,8	1,0	4,3	3,8	$3,7^{B}$
	Testosterone	5,8	1,8	5,3	3,8	$4,2^{B}$
	Mean	$5,8^{a}$	$2,3^{b}$	$5,3^{a}$	$5,4^{a}$	-
Callus formation rate at the end of the 30th day (%)	**Progesterone**	$26,6^{c}$	$56,3^{a}$	$45,3^{ab}$	$34,4^{b}$	$40,6^{BC}$
	Estrone	$26,6^{d}$	$84,4^{a}$	$45,3^{c}$	$60,9^{b}$	$54,3^{A}$
	17β-estradiol	$26,6^{c}$	$40,6^{bc}$	$51,6^{ab}$	$64,1^{a}$	$45,7^{AB}$
	Testosterone	$26,6^{b}$	$53,1^{a}$	$26,6^{b}$	$25,0^{b}$	$32,8^{C}$
	Mean	$26,6^{c}$	$58,6^{a}$	$42,2^{b}$	$46,1^{b}$	-
Viability rate at the end of 90th day (%)	**Progesterone**	$34,4^{b}$	$73,4^{a}$	$73,4^{a}$	$46,9^{b}$	$57,0^{A}$
	Estrone	$34,4^{a}$	$46,9^{a}$	$42,2^{a}$	$40,6^{a}$	$41,0^{B}$
	17β-estradiol	$34,4^{b}$	$21,9^{b}$	$60,9^{a}$	$57,8^{a}$	$43,8^{B}$
	Testosterone	$34,4^{ab}$	$18,8^{b}$	$42,2^{a}$	$32,8^{ab}$	$32,0^{C}$
	Mean	$34,4^{b}$	$40,2^{b}$	$54,7^{a}$	$44,5^{ab}$	-
Number of shoots rooted at the end of 90th day (shoot /petri)	**Progesterone**	1,5	0,5	3,0	2,0	1,8
	Estrone	1,5	1,3	2,5	0,8	1,5
	17β-estradiol	1,5	0,0	3,0	3,0	1,9
	Testosterone	1,5	0,3	2,8	1,5	1,5
	Mean	$1,5^{bc}$	$0,5^{c}$	$2,8^{a}$	$1,8^{ab}$	-
Number of shoots at the end of 90th day (shoot /petri)	**Progesterone**	$1,8^{b}$	$4,4^{a}$	$4,1^{a}$	$2,8^{a}$	$3,3^{A}$
	Estrone	$1,8^{c}$	$3,3^{a}$	$2,6^{b}$	$2,9^{b}$	$2,7^{B}$
	17β-estradiol	$1,8^{c}$	$1,6^{c}$	$4,7^{a}$	$2,5^{b}$	$2,7^{B}$
	Testosterone	$1,8^{b}$	$0,7^{b}$	$2,3^{a}$	$1,3^{b}$	$1,5^{C}$
	Mean	$1,8^{b}$	$2,5^{b}$	$3,4^{a}$	$2,4^{b}$	-

1) The difference between the means shown at the same column with the same capital letter and at the same line with the same lower-case letter are insignificant

Effects of MSH on the root-forming capacity of hypocotyl. Hormone types, hormone doses, hormone x dose interaction had an important effect ($p<0,01$) on root-forming capacity of hypocotyl (at the end of 30th ve 60th days) (Table 4). When the mean value of hormone types given in Table 4 analyzed, it was seen that when hypocotyl was used as explant, testosterone took in the first place according to the ranking root-forming explants (8,5 root/petri). 4,8 roots were generated in the control. When root generation analyzed according to days testosterone with 10,3 roots took the first place. Increase number of days had a positive effect on results (Table 4).

Effects of MSH on the rooting capacity of root explant's. The differences of hormone doses and hormone types related to root explant's capacity of generating root were statistically very important ($p<0,01$) (Table 5). When root was used as explant, progesterone was the first with 7,0 roots followed by; the control group with 5,8 roots according to the mean of explants generating root. When 12

combinations created were examined separately according to hormone types ans doses, the most root number was obtained from progesterone's 10^{-6} mM dose (9,3) and the other doses of progesterone followed 10^{-5} mM (7,3) and 10^{-4} mM (5,5) (Table 5).

Effects of MSH on the callus formation rates of root. The differences that belong to hormone doses and hormone types and hormone X dose interaction had an important effect on the rate of root's callus formation. ($p<0,01$) (Table 5). When the mean values of hormone types were considered, estrone had the highest amount of the callus formation (54,3 %). The rate of callus formation in the control was 26,6 %. According to the mean of hormones doses, 10^{-4} mM dose (58,6 %) ranked first in terms of the callus formation (Table 5). When hormone types were examined separately according to applied doses, estrone's 10^{-4} mM dose (84,4 %) had the highest rate of callus formation.

Effects of MSH on the viability rates of root. The differences of hormon types and doses in the viability rate of the root explants were statistically very important. In a similar way, hormone x dose interaction also became significant (Table 5). When the mean values related to hormone types were analyzed, the highest viability rate was obtained from progesterone (57,0 %). However, it was seen that the highest viability rate (54,7 %) belonged to 10^{-5} mM dose when the means of all the doses of the hormones were analyzed. As it is understood from data, viability rate of the mean of all the hormone doses was higher than the control group (Table 5). When hormone types were examined separately according to the applied doses, progesterone's $10^{-4}$and 10^{-5} mM doses (73,4 %) had the highest rate of viability (73,4 %). Besides, 17β-estradiol's 10^{-5} and 10^{-6} mM doses had an important effect on the viability (Table 5).

Effects of MSH on number of rooted-shoot taken from root. The differences of doses related to rooting of shoots taken from root explant were statistically very important (Table 5). When the mean values of the hormone types were considered, 17β-estradiol with (1,9) roots took in the first place, progesterone followed it with (1,8) roots in terms of rooting of shoots taken from root explant. When the hormone types were examined separately according to the applied doses, progesterone's 10^{-5} mM dose with (3,0) roots, 17β-estradiol's 10^{-5} mM and 10^{-6} mM doses were in the first place. When the mean of the hormone doses was examined, it was determined that 10^{-5} mM dose with (2,8) roots took the first place (Table 5).

Effects of MSH on shoot forming capacity of root. When the results of variance analysis related to the generated shoot explant at the end of 30th 60th and 90th days were examined, it was seen that the doses had very important effect at the end of 30th day. Hormones and doses had very important effect at the end of 60th and 90th days ($p<0,01$). Also, hormone X dose interaction's effect was very important at the end of 90th day ($p<0,01$) (Table 5).

The mean values given in Table 6 show that progesterone took the first place with 3,3 in terms of shoot-forming explants. On the other hand, shoot-forming explants number was 1,8 in control group. When the hormone types were examined separately, it was found that when the number of applied day increased, the number of shoots also increased. And, in terms of that feature the most number shoots (6,0) were taken from sainfoin's root explants that placed in the medium including progesterone for 90th days (Table 6). According to the mean of the applied doses, 10^{-5} mM dose with 3,4 shoots took in the first place. When the values related to dose and days were examined separately, it was seen that 10^{-5} dose (5,6) gave better results. It was seen that increase number of applied days the number of shoot also increased (Table 6).

TABLE 6
Number of shoot-forming explants at the end of 30th, 60th and 90th day according to different hormone types

		Number of treatment days			
		30	**60**	**90**	**Mean**
Hormone	**Progesterone**	$1,0^{a}$	$2,9^{a}$	$6,0^{a}$	$3,3^{A}$
	Estrone	$1,1^{a}$	$2,7^{a}$	$4,3^{b}$	$2,7^{AB}$
	17β-estradiol	$1,0^{a}$	$2,8^{a}$	$4,2^{b}$	$2,7^{AB}$
	Testosterone	$0,4^{b}$	$1,6^{b}$	$2,6^{c}$	$1,5^{B}$
	Mean	0,9	2,5	4,3	-
Doses (mM)	**0**	$0,3^{b}$	$2,0^{b}$	$3,3^{b}$	$1,8^{B}$
	10^{-4}	$1,0^{a}$	$2,2^{b}$	$4,3^{ab}$	$2,5^{B}$
	10^{-5}	$1,3^{a}$	$3,4^{a}$	$5,6^{a}$	$3,4^{A}$
	10^{-6}	$0,9^{a}$	$2,3^{b}$	$3,9^{b}$	$2,4^{B}$
	Mean	1,0	2,6	4,5	-

1) The differences between the means shown at the same column with the same capital letter and at the same line with the same lower-case letter are insignificant.

TABLE 7
Number of shoot forming explant at the end of 90th day according to different hormone types and doses

Hormone	Doses (mM)				
	0	10^{-4}	10^{-5}	10^{-6}	Mean
Progesterone	$1,8^{b}$	$4,4^{a}$	$4,1^{a}$	$2,8^{a}$	$3,3^{A}$
Estrone	$1,8^{c}$	$3,3^{a}$	$2,6^{b}$	$2,9^{b}$	$2,7^{B}$
17β-estradiol	$1,8^{c}$	$1,6^{c}$	$4,7^{a}$	$2,5^{b}$	$2,7^{B}$
Testosterone	$1,8^{b}$	$0,7^{b}$	$2,3^{a}$	$1,3^{b}$	$1,5^{C}$
Mean	$1,8^{b}$	$2,5^{b}$	$3,4^{a}$	$2,4^{b}$	-

1) The differences between the means shown at the same column with the same capital letter and at the same line with the same lower-case letter are insignificant.

When the combinations consisting of hormone types and doses were analyzed separately, it was found that 17β-estradiol's 10^{-5} mM'dose with 4,7 shoots took the first place, progesterone' s 10^{-4} and 10^{-5} mM doses followed it with 4,4 shoots and 4,1 shoots respectively. It was observed that the lowest effect level on the shoot generation belonged to testosterone (Table 7).

DISCUSSION

In tissue culture studies, the most important things to consider type and concentration of plant growth regulators and choosing appropriate explant. It is reported as a results of the studies on many plants including sainfoin if auxin and cytokinin bring into balance in a desired way, there will be wide range of adventive shoot regeneration [11, 12, 13, 14, 15, 16, 17].

Up to now, many researches on sainfoin have been conducted and various different topics have been examined. However, the researches related to mamal steroid sex hormones are very few. This study aimed at determining effects of certain mamal sex hormones on callus formation and plant regeneration in sainfoin. Thanks to the results obtained, it can be said that effects of hormone types vary according to explant types. In terms of hormones, 17β-estradiol had the most impact on shoot generation capacity of cotyledons; estrone on shoot generation from hypocotyl and progesterone on shoot generation from root. In other respects, shoot generation varied in each of three explant types according to hormone doses. It was deduced that 17β-estradiol and progesterone's 0,25 µg dose increased shoot generation (per plant) in sunflower seedling. (*Helianthus annus* L.) [18]. Additionaly, it was found that the number of shoots generated from roots varies according to the number of application day, and it was seen that number of shoots also increased as the number of application day increased. There have not been any studies undertaken in the area of the effects of the mamal steroid sex hormones on viability rate of cotyledons and roots. In this respect, it is thought that this is the first study on the effects of MSH on viability rate of cotyledons and roots. The highest viability rate was determined in the mediums including estrone for cotyledons, in the mediums including progesterone for root explant. When cotyledons were used as explant, no callus was formed. On the other hand, when hypocotyl were used as explant, callus was formulated and the highest callus formation rate was determined in the mediums including progesterone, testosterone, 17β-estradiol and estrone followed it. Besides, there was no callus formation in the control group in which there was no hormone. According to this fact, it was understood that in cases where hypocotyl used as explant mamal steroid sex hormones triggered callus formation. Callus was formed from root mostly in the mediums including 17β-estradiol and estrone. In another study performed on *Polygonatum verticillatum*, it was determined that 17β-estradiol increased callus formation [19, 20]. In a study on *Arabidopsis thaliana*, it was stated that androstenedion triggered callus formation. Also, it was understood that in wheat androsterone and androstenedion (1 µM) triggered the callus tissue in scutellum and increased the growing and germination of immature embryo. In addition to this, it was seen that estrogenes, especially estrone inhibited germination of immature embryo but it did not inhibit the callus formation [7, 19, 21, 22]. Also, in the other studies on *Polygonatum verticillatum*, it was revealed that 17β-estradiol enhanced the formation and reproduction of callus [23, 24]. In the study immature wheat embryo were used, Janeczko et al. [21] were observed that androsterone and testosterone were increased callus formation, estrone and progesterone inhibited the callus formation.

As a result of the studies conducted on mamal steroid sex hormones, these were found that it increased the plant growing in trefoil Shore et al. [25], Şahin et al. [20], accelerated the speed of shoot generation in sunflower [18], accelerated the speed of generative growing in wheat Janeczko and Filek [26], and increased the plant growing in corn [27].

In this study, testosterone took the first place according to the effects of hormone types on hypocotyl's root generation, 17β-estradiol took the second place. Additionaly, when the root was used as explant, progesterone was the first place, the control group followed it. In terms of the effect on rooting the shoots taken from root 17β-estradiol took the first place, progesterone followed it. Afterall, 17β-estradiol and progesterone's 0,25 µg dose (per plant)

inhibited the root development of seedlings of sunflower (*Helianthus annus* L.) in the study conducted by Bhattacharya and Gupta [18]. Progesterone's 0,1 µg dose (per plant) had a positive effect on increase in length of root and testosterone's 0,1 and 0,25 µg doses (per plant) increased the formation of bud were observed. Both results of that study and results of the previous studies showed that effects of different hormones varied according to explants. As mentioned above, there is lack of information related to mechanism of action because the number of the study on using mamal steroid sex hormones in tissue culture is rather limited. Results of this study match up with the results of previous studies.

The experiments that were established to determine the effects of mamal steroid sex hormones on adventive shoot regeneration in sainfoin which is an important forage legume. Cotyledon, root and hypocotyl explants taken from *in vitro* seedlings were cultured on the medium including mamal steroid sex hormones with different doses. This is the first research on sainfoin that using mamal steroid sex hormones under the tissue culture condition and positive results was obtained in terms of its effect on callus formation and regeneration. Using mamal steroid sex hormones in plant tissue culture is pretty new application. Therefore, further studies must be conducted on different plants by using same or different hormone types and doses to clearly understand the effects of them on callus and regeneration. Besides a further research will be conducted in order to provide insights into mechanisms of action in tissue culture.

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