Effects of treating with auxin solutions on rooting of cuttings of sainfoin (*Onobrychis viciifolia*)

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Abstract

Sainfoin is a cross-pollinating plant and segregates when material is multiplied from seed during breeding programs. While the use of cuttings allows rapid and reliable multiplication of selected material for vegetative propagation, cuttings often do not root well. This study examined the root development of sainfoin cuttings after dipping in solutions containing 0, 25, 50, 100 and 200 mg/L indole-3-acetic acid (IAA), α–naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) for 8, 16 and 24 h. The hormones were dissolved in either liquid Murashige and Skoog (MS) medium or distilled water. While untreated cuttings produced virtually no roots, all auxin treatments stimulated rooting on some cuttings. Dipping treatments using liquid MS medium as the base for the auxin solution produced better root development than those using distilled water. IBA was less effective than IAA or NAA in stimulating root production on cuttings. Dipping cuttings in a solution of IAA at 100 mg/L for 8 h in liquid MS medium would seem to be adequate to produce satisfactory root development. Rooted plantlets were transferred to pots and grew successfully under greenhouse conditions.

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Introduction

The *Onobrychis* genus encompasses about 162 species and is native to Anatolia, Caucasus and Iran (Aktoklu 1995). Sainfoin (*Onobrychis sativa* Lam or *Onobrychis viciifolia* Scop.) is the most widespread species of this aggregate (Çeliktaş *et al.* 2006), having spread from the Baltic to the Mediterranean, and from the Atlantic to the Himalayas. Sainfoin is a cross-pollinated perennial legume used for hay and forage in dry regions of central and eastern Anatolia. It has a deep tap root that makes it drought-tolerant, and it grows well on calcareous soils (Elçi 2005). It contains condensed tannins, which reduce its potential to produce bloat and improve protein digestion by grazing animals (Rumball and Claydon 2005). The flowers produce large amounts of nectar, so it is commonly used for honey production (Elçi 2005). These characteristics have made it a crop of interest to scientists and farmers in recent years.

Breeding studies with sainfoin to increase both yield and quality of forage and resistance to insect pests are underway (Elçi *et al*. 1996). Progress is hampered by the cross-pollinating nature of sainfoin. An easy way of producing abundant and reliable supplies of desirable plants in a short time, while maintaining genetic stability, is to use vegetative propagation.

Ex vitro vegetative propagation using cuttings is common practice for multiplying many plant species (Eliasson and Areblad 1984; Sevimay *et al.* 1994; Syed *et al.* 2002; Abdullah *et al*. 2005; Danehloueipour *et al*. 2006; Henrique *et al*. 2006). Cuttings from some species root readily without auxin treatment, while cuttings from others do not root easily (Eliasson and Areblad 1984; Griffith 1998; Hartmann *et al*. 2002; Blythe *et al*. 2004). There are many studies about *in vitro* propagation and rooting of plantlets from sainfoin hypocotyls, cotyledons, leaves, stems, petioles and immature and mature embryo explants using MS medium + 30 g/L sugar containing

various auxins (Ozcan *et al*. 1996; Ozgen *et al*. 1998; Sancak 1999; Çeliktaş *et al*. 2006). However, some difficulties exist such as optimisation of sterilisation, tissue culture and acclimatisation procedures and the process is time-consuming. Use of *ex vitro* techniques has not been reported to our knowledge. *In vitro* rooting studies have shown that use of auxin solutions based on MS medium is more efficient than pure auxin application. This study aimed to establish a method for simple and reliable *ex vitro* propagation of sainfoin using cuttings.

Materials and methods

Sainfoin seeds were obtained from General Directorate of Agricultural Enterprises, Turkey, and were sown in experimental fields of the Department of Field Crops, Faculty of Agriculture, Ankara University in September 2006. Cuttings (5–6 cm long stem pieces including apical meristems) containing 2 or 3 nodes were harvested from these field-grown plants in May 2008. Using a factorial design, cuttings were dipped in solutions of 0, 25, 50, 100 and 200 mg/L indole-3-butryic acid (IBA), indole-3-acetic acid (IAA) and α–naphthalene acetic acid (NAA) for 8, 16 and 24 h before being planted in perforated plastic trays containing perlite and kept in the greenhouse. The auxin solutions were prepared using either: liquid MS medium (Murashige and Skoog 1962) containing MS basal salt mixture and vitamins (Sigma no. M-5519) plus 30 g/L sugar; or distilled water. The pH was adjusted to 5.6–5.8. Control cuttings remained untreated. The cuttings were watered daily to maintain sufficient moisture. The perforated plastic trays were kept in a growth chamber at 16±1˚C with 16 h light and 8 h darkness and 60% relative humidity. Fifteen days later, the number of cuttings with roots, the number of roots per cutting and the length of the longest root on each cutting were recorded. Rooted cuttings were then transferred to pots containing sand, clay and peat in equal proportions and grown out in the greenhouse for 2 months.

The experiments were conducted in a split-split-plot design with a factorial arrangement using 3 replications. Auxin treatments were main-plots, while auxin concentrations were sub-plots and dipping durations were sub-sub-plots. Each replication consisted of 10 cuttings. Data given in percentages were subjected to arcsine transformation before statistical analysis (real data are given in Tables 1 and 2). Analyses of variance for all investigated parameters were performed using SPSS software (Windows Version 11). The differences among means were tested using the LSD test ($P<0.05$).

Results

Tables 1 and 2 show the effects of auxins applied in liquid MS medium plus 30 g/L sugar (Treatment 1) or in distilled water (Treatment 2) on percentage of cuttings which developed roots, the number of roots per cutting and the length of the longest root. There was a significant interaction between treatments ($P<0.05$), so results for the two different media are presented separately.

Very little rooting was recorded on untreated stem cuttings. When liquid MS medium was used as the base for the auxin solutions, dipping in IAA, NAA and IBA resulted in rooting frequencies in the ranges of 0–77%, 0–50% and 0–33%, respectively. More than 70% of cuttings produced roots when dipped in IAA at 100 or 200 mg/L for 8 hours (Table 1).

Dipping in IBA was less effective in stimulating root production than dipping in either IAA or NAA, with fewer cuttings producing roots and the number of roots per cutting being lower. While there was a tendency for root numbers and root length to increase as concentration of auxin increased, concentrations of 50 mg/L usually gave close to optimum results (Table 1).

In general, auxins mixed with distilled water were less effective in stimulating rooting of cuttings than auxins in liquid MS medium (Table 2). Again IBA tended to be less effective than IAA and NAA in stimulating root production.

When rooted cuttings were transferred to pots and grown out in the greenhouse, all grew successfully.

Discussion

This study has shown that auxin type, concentration and duration of application proved important factors in vegetative propagation of sainfoin. While untreated cuttings produced virtually no roots, all auxin treatments produced root growth and the trend was for increased percentage of cut-

Table 1. Effects of auxin type, concentration and duration of dipping on rooting percentage, root number and root length on sanfoin cuttings using liquid MS as base.

Auxin type (AT)[1]	Auxin concentration (AC, mg/L)	Rooting rate (%)			Root number per cutting			Length of longest root (cm)		
		Duration (D)			Duration			Duration		
		8 h	16 h	24 h	8 h	16 h	24 h	8 h	16 h	24 h
IAA	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	25	13.33	13.33	16.67	1.33	1.00	3.66	4.35	7.06	8.86
	50	46.67	3.33	46.67	4.33	0.67	6.67	9.67	1.75	10.04
	100	73.33	20.00	43.33	11.33	7.67	20.33	13.99	8.85	14.04
	200	76.67	66.67	46.67	17.00	12.00	7.00	12.73	10.56	6.72
NAA	0	0.00	0.00	6.67	0.00	0.00	3.33	0.00	0.00	6.17
	25	3.33	23.33	20.00	0.67	8.33	2.33	4.51	16.99	5.56
	50	40.00	40.00	50.00	11.67	11.67	16.33	12.64	12.59	12.97
	100	33.33	26.67	33.33	12.00	24.00	17.66	8.59	14.99	8.97
	200	16.67	46.67	30.00	3.33	7.33	17.33	4.13	6.34	8.11
IBA	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	25	10.00	3.00	0.00	2.33	0.67	0.00	3.85	2.35	0.00
	50	10.00	0.00	0.00	3.33	0.00	0.00	9.35	0.00	0.00
	100	6.67	13.33	20.00	7.00	11.33	3.33	10.09	12.52	7.24
	200	23.33	33.33	20.00	9.33	5.67	10.67	10.13	7.17	10.63
		AT*AC*D LSD=15.48 (P<0.05, df=90)			AT*AC*D LSD=7.761 (P<0.05, df=90)			AT*AC, LSD=3.830 AT*D, LSD=2.967 AC*D, LSD=3.830 (P<0.05, df=90)		

[1]IAA = indole-3-acetic acid; NAA = α–naphthalene acetic acid; IBA = indole-3-butyric acid.

tings producing roots, with more and longer roots per cutting, with increasing concentration of auxin. However, there seems little to be gained in most situations from using concentrations above 100 mg/L for 8 h. Overall, IAA seemed to be the superior auxin to use as reported by De Klerk *et al*. (1997), Thirunavonkkarasu and Saxena (1997), Soyler and Arslan (2000) and Kan *et al*. (2002).

In general, auxins mixed with liquid MS medium plus 30 g/L sugar were more effective in stimulating rooting of stem cuttings than auxins mixed with distilled water. The micro and macro elements and vitamins in the liquid MS medium might have stimulated rooting, producing the superior results over that with distilled water-based solutions. Other authors (Turetskaya and Polikarpova 1968; Christov and Koleva 1995) have reported increased root initiation on cuttings of some plants when vitamin C, B_1 or K was added to the solution of growth regulators. Rupela and Dart (1981) and Syed *et al.* (2002) suggested that application of Hoagland solution could increase rooting success, while MS basal salt mixture and vitamins have been mostly used in *in vitro* rooting studies (De Klerk *et al*. 1997; Rani *et al*. 2008).

Based on these results, it is concluded that:

- Dipping in auxin solutions can stimulate root production by sainfoin cuttings.
- IBA is less effective in this regard than IAA and NAA.
- Liquid MS + 30 g/L sucrose seems preferable to distilled water as the base for preparing the auxin solutions.
- Dipping in a solution of 100 mg/L IAA for 8 h appears adequate to obtain satisfactory root development of sainfoin cuttings in perlite.

Application of this technology would allow rapid multiplication of material in sainfoin breeding programs without risk of change of

Table 2. Effects of auxin type, concentration and duration of dipping on rooting percentage, root number and root length on sainfoin cuttings using distilled water as base.

Auxin type (AT)[1]	Auxin concentration (AC, mg/L)	Rooting rate (%)			Root number per cutting			Length of longest root (cm)		
		Duration (D)			Duration			Duration		
		8 h	16 h	24 h	8 h	16 h	24 h	8 h	16 h	24 h
IAA	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	25	6.67	23.33	0.00	1.00	3.00	0.00	2.46	4.32	0.00
	50	16.67	30.00	30.00	2.33	3.67	9.00	5.26	4.65	5.38
	100	26.67	36.67	33.33	8.33	7.67	8.33	5.77	8.55	5.37
	200	40.00	63.33	23.33	11.66	10.00	9.67	8.21	7.76	4.46
NAA	0	0.00	0.00	6.67	0.00	0.00	3.33	0.00	0.00	6.17
	25	6.67	3.33	6.67	0.67	1.00	1.67	1.89	1.74	1.06
	50	10.00	0.00	23.33	3.00	0.00	5.00	5.32	0.00	5.61
	100	0.00	40.00	16.67	0.00	7.67	3.33	0.00	7.77	4.29
	200	33.33	33.33	30.00	6.67	8.33	7.33	4.37	7.36	5.77
IBA	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50	0.00	3.33	3.33	0.00	2.00	4.00	0.00	2.91	1.36
	100	3.33	30.00	0.00	2.67	6.67	0.00	2.99	7.65	0.00
	200	16.67	26.67	6.67	3.33	6.33	12.00	2.79	7.89	5.43
		AT*AC*D LSD=14.19 (P<0.05, df=90)			AT, LSD=1.419 AC, LSD=1.832 (P<0.05, df=90)			AT*D, LSD=1.769 AC*D, LSD=2.283 (P<0.05, df=90)		

[1]IAA = indole-3-acetic acid; NAA = α–naphthalene acetic acid; IBA = indole-3-butyric acid.

genetic makeup of plants produced through cross-pollination, and preservation of agriculturally valuable characteristics of selected materials. Further studies could be conducted to clarify the optimum concentration of IAA to use and the optimum duration of dipping.

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