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GROWTH REGULATORS EFFECTS ON *IN VITRO* SHOOT REGENERATION OF SAINFOIN (*ONOBRYCHIS SATIVA* LAM.)

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ABSTRACT

The effect of different concentrations of BAP (6-benzylaminopurine)-NAA (a-naphthaleneacetic acid) on in vitro propagation from cotyledon node explant of sainfoin (Onobrychis sativa Lam.) was studied. The developed large numbers of shoots were propagated from explants within 6 weeks on Murashige and Skoog (MS) media. The longest shoot length (6.79 cm) was recorded on MS medium containing 1 mg/l BAP, 0.02 mg/l NAA. The observed difficulties were overcome after pulse treatment with 50 mg/l IBA for 10 min on MS medium for rooting.

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Keywords: Onobrychis sativa, Sainfoin, shoot regeneration, cotyledon node, BAP, NAA

Introduction

Sainfoin (*Onobrychis sativa* Lam.) is an important Eurasian perennial forage legume crop and includes over 150 species of which 53 species are found in Turkey alone (17). *O. sativa* is well adapted to highland farming system under Anatolian dry land conditions and also is reported as none bloating and highly nutritional crop plant in the local farming system. These crops are also grown in Europe as far as Sweden and grow well on all type of lands including wastelands. They fix atmospheric nitrogen and improve soil organic matter. Sainfoins with pale pink flowers, are also used by honey bees for pollination and production of honey. The crops produce highly nutritious forage and can be used judiciously by heavy working animals in agriculture. Moreover, these plants have a deep root system, which make them highly drought resistant.

Root boring insects (*Bembecia scopigera* and *Sphenoptera carceli*) tunnel into the main central part of the root and kill the plant before seeds can be set up. The plant is losing importance in the agricultural systems of Turkey after it has lasted so many years. Classical methods of breeding have failed to produce resistant crops which overcome this problem. Therefore, breeding activities need to be supplemented through modern biotechnological means. Previous reports suggest shoot regeneration from intact embryonic axes, immature embryos, leaflets, petioles and stems (10, 11, 12, 14) but there has been no report of shoot regeneration from cotyledon node explant in sainfoin. Tissue culture and genetic transformation could be possibly used to improve the plant and obtain plants that are resistant against theses insects and other problems related to the insect through *Agrobacterium* mediated genetic transformation. Therefore, it was considered important to develop and optimize shoot regeneration and rooting protocol that could be effectively used for further Agrobacterium mediated transformation experiments. Improvement of this crop by genetic engineering techniques will have great impact on the future of this plant for use in all areas of the world where it is used for feeding of animals.

Materials and Methods

Seeds of sainfoin were obtained from the Department of Agronomy, Faculty of Agriculture, Ankara University, Ankara, Turkey. The seeds were surface sterilized with 50% commercial bleach (Ace, Turkey, containing 5% NaOCl) for 20 min followed by 3 x 5 min rinsing with bidistilled sterilized water. The seeds were cultured for germination on 35 ml of agar solidified MS basal medium (8) supplemented with 3.0% sucrose in 100 x 10 mm Petri dishes. Agar (0.65%- Duchefa Biochemie B.V., Haarlem, The Netherlands) was added to the culture medium after adjusting pH to 5.6-5.8 before autoclaving at 121^{o}C for 20 minutes.

Cotyledon node explants were excised from 7-8 days old *in vitro* grown seedlings. They were cultured on 35 ml MS medium containing 0.5, 1.0 mg/l 6-Benzylaminopurine (BA, Cat No. B3408, Sigma Aldrich Chemical Co., St. Lo. Mo.) and 0, 0.01, 0.02 mg/l NAA, 3.0% sucrose gelled with 0.65% agar. The pH of all medium was adjusted to 5.6-5.8 using 0.1 N KOH or 0.1 N HC1 before sterilization and solidification by 0.65% agar. All cultures were incubated in growth chamber (Sanyo E&E Europe BV, Biomedical Division, UK) at 24±2^{o}C with 16 h light photoperiod.

The longest shoots were regenerated as both explants were excised under aseptic conditions after six weeks of regeneration. They were pulse treated with 50 mg/l IBA (indole 3 butyric acid, Cat No. I5386, Sigma Aldrich Chemical Co., St. Lo. Mo.) for 10 min and then rooted on 35 ml of MS in Magenta GA7 vessels for four weeks. Thereafter, agar was carefully removed from the roots under tap water. Following, half plants were submerged in water for 15-20 min to avoid wilting of leaves and in order that turgor was maintained, and the other half were planted directly into the soil. The tissue

cultured plants were transferred to pots containing vermiculite, organic matter and sand (1:2:1) in the greenhouse at room temperature, where plants were subjected to intermittent mist-water spray with HR-15 Cool Mist humidifier with humidistat (Devon, UK) turned on for 24 hours. Relative humidity was kept at 90% during the first few days, which helped to maintain a film of water on the plant leaves in order that wilting was avoided. The humidity was gradually reduced to 40% in two weeks.

All treatments of regeneration experiments had three replicates containing five explants each and all experiments were repeated twice (3x5x2=30 explants per treatment). Data for frequency (%) of shoot regeneration, mean number of shoots per explant, shoot length and frequency of rooting was recorded and analyzed using one way ANOVA with statistical software SPSS 16.00 for windows. The post hoc tests were performed using Duncans Multiple Range Test. Data given in percentages were subjected to arcsine transformation (16) before statistical analysis.

Results and Discussion

Optimization of shoot regeneration

The explants began to swell within 7-8 days of culture, followed by development of shoot meristems, shoot initials and subsequently by shoot in four weeks. These developed into full shoots after 6 weeks of culture.

Shoot regeneration was recorded on all explants irrespective of the concentration of BAP and NAA in the MS regeneration media (**Table 1**). However, axillary shoot regeneration range varied and the number of shoots per explant significantly varied baseed on the concentration of plant growth regulators in the MS medium. Sharp fluctuations number of shoots per explant were recorded on MS regeneration medium at 0.5 mg/l BAP with variants of NAA and 1 mg/l BAP with variant of NAA. The shoots showed well developed green leaves with maximum number of shoots on medium containing 0.5 mg/l BAP. Addition of 0.01 or 0.02 mg/l NAA in the medium was inhibitory and resulted in induction of significantly reduced number of shoot. MS medium containing 1 mg/l BAP singly was inhibitory and resulted in minimum number of shoots per explant. However each increase in the concentration of NAA with 1 mg/l BAP resulted in increased shoot regeneration with maximum shoot regeneration on MS medium containing 1 mg/l BAP and 0.02 mg/l NAA.

Developing shoots considerably increased in length in 6 weeks time. The longest shoots (6.79 cm) were recorded on MS medium containing 1 mg/l BAP, 0.02 mg/l NAA followed closely by 5.97 cm long shoots on MS medium containing 1 mg/l BAP, 0.01 mg/l NAA.

Well developed shoots regenerated on MS medium containing 1 mg/l BAP and 0.02 mg/l NAA were excised and pulse treated with 50 mg/l IBA for 10 min and then cultured on MS medium for rooting. No difficulty was observed on rooting of pulse treated shoots. The results showed that the plants that were submerged in water for 15 to 20 min were easy to acclimatize compared to the plants that were not submerged in water and planted directly into the pots. The results showed 100% establishment of plants that were submerged in water before potting. The plants that were not submerged in water before potting showed 40% establishment in the greenhouse. All acclimatized plants grew normally and set seed in the greenhouse.

Due to the outbreeding nature of sainfoin, plants propagated by sexual methods are highly heterogeneous and therefore, asexual (vegetative) propagation has great importance for preserving uniformity and unique characteristics of this crop (5). Probably, the most widespread technique for vegetative propagation is reproduction by actively growing pieces of shoots, called cuttings (10). This study reports effects of varying concentrations of BAP used singly or in combination with NAA in the regeneration media on *in vitro* micropropagation from cotyledon node explant of sainfoin.

In vitro micropropagation of sainfoin has been reported previously by Ozcan et al. (11, 12). They obtain shoot regeneration from intact embryonic axes, immature embryos and leaflets, petioles and stems. However, no report signify the importance of cotyledon node for use in the shoot regeneration experiments. The results of this study clearly reveal the importance of cotyledon node in micropropagation of sainfoin. The results show that optimal combinations of BAPand NAA in the MS medium could play effective role in enhancing shoot

TABLE 1

Effects of different concentrations of BAP-NAA on shoot regeneration from cotyledon node explant of sainfoin

Medium		Frequency of shoot regeneration (%)	Number of shoots per explant	Shoot length (cm)
BAP (mg/l)	NAA (mg/l)			
0.5	0.00	100	16.55 a	2.33 e
0.5	0.01	100	14.92 b	3.45 d
0.5	0.02	100	14.67 b	4.36 c
1.00	0.00	100	12.67 c	2.54 e
1.00	0.01	100	13.83 bc	5.97 ab
1.00	0.02	100	16.43 a	6.79 a

values within a column followed by different letters are significantly different at 0.05 probability level using Duncan's multiple range test

multiplication capacity of cotyledon node explants with effect on both shoot multiplication and shoot length. In general, high BAP levels (1 mg/l) with 0.02 increased the number of shoots per explant and shoot length. The results are in agreement with shoot regeneration from immature embryos in *Centaurea tchihatcheffii* (9), cotyledon node in cowpea (1), T*hymbra spicata* (4), Hungaria vetch (13), Narbon vetch (6), wild lentil (15) and lentil (7) and using different cytokinin and auxins in the MS regeneration medium.

The rooting of the plants was not difficult; however, the plantlets showed variations as per pre-treatment during acclimatisation procedure. The plants that were submerged for 15-20 minutes in tap water were not difficult to acclimatise. However, those not submerged in water, showed wilting with acclimatisation rate of 40% only. The results are in agreement with previous studies by Aasim et al. (2, 3) in cowpea; the authors treated their explants by submerging in water before acclimatization.

Conclusions

All experiments were completed in 12 weeks. The results confirm that cotyledon node can be effectively used for rapid microproapagation of sainfoin in a short period of time and can be effectively used for micropropagation of the plant.

REFERENCES

1. **Aasim M., Khawar K.M., Ozcan S.** (2008) Bangladesh J. Bot., **37**(2), 149-154.
2. **Aasim M., Khawar K.M., Ozcan S.** (2009a) Scientia Horticulturae, **122**, 468-471.
3. **Aasim M., Khawar K.M., Ozcan S.** (2009b) Not. Bot. Hort. Agrobot. Cluj, **37**(1), 89-93.
4. **Daneshvar-Royandezagh S., Khawar K.M., Ozcan S.** (2009) Biotechnology & Biotechnological Equipment, **23**(3), 1319-1321.
5. **George E.F. and Sherrington P.D.** (1984) Plant propagation by tissue culture, Basingstoke, Exegetics Ltd., Westbury.
6. **Kendir H., Sahin-Demirbag N., Khawar K.M., Aasim M.** (2008) African Journal of Biotechnology, **7**(14), 2491-2494.
7. **Khawar K.M. and Ozcan S.** (2002) Biotechnology & Biotechnological Equipment, **16**(1), 12-17.
8. **Murashige T. and Skoog F.** (1962) Physiol. Plant., **15**, 473- 497.
9. **Ozel C.A., Khawar K.M., Mirici S., Ozcan S., Arslan O.** (2006) Naturwissenschaften, **93**, 511-517.
10. **Ozcan S., Yildiz M., Sancak C., Ozgen M.** (1996) Tr. J. of Botany, **20**, 497-501.
11. **Ozcan S., Sevimay C.S., Yildiz M., Sancak C., Ozgen M.** (1996) Plant Cell Rep., **16**, 200-203.
12. **Ozgen M., Ozcan S., Sevimay C.S., Sancak C., Yildiz M.** (1998) Plant Cell, Tiss. Org. Cult., **52**, 205-208.
13. **Sahin-Demirbag N., Kendir H., Khawar K.M., Aasim M.** (2008) Biotechnology and Biotechnological Equipment, **22**(4), 929-932.
14. **Sancak C.** (1999) Tr. J. of Botany, **23**, 133-136.
15. **Sevimay C.S., Khawar K.M., Yuzbasioglu E.** (2005) Biotechnology and Biotechnological Equipment, **19**(2), 46-49.
16. **Snedecor G.W. and Cochran W.G.** (1967) Statistical Methods, The Iowa State Univ. Press, Iowa. pp 527-529.
17. **Tubives** (2010) http://www.tubitak.gov.tr/tubives