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KHALID MAHMOOD KHAWAR

SEBAHATTİN ÖZCAN

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Research Note

Effect of Indole-3-Butyric Acid on *in vitro* Root Development in Lentil (*Lens culinaris* Medik.)

Khalid Mahmood KHAWAR, Sebahattin ÖZCAN
Department of Field Crops, Faculty of Agriculture, University of Ankara, Dışkapı, Ankara - TURKEY

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Abstract: Lentil is an important crop of the family *Leguminosae* and is notoriously recalcitrant to rooting *in vitro*. Shoots of cultivar "Ali Dayı" of lentil obtained after culturing seeds for 10 days on MS medium were isolated and rooted on MS medium containing indole-3-butyric acid (IBA) at concentrations of 0.25, 0.5, 1.0 and 2.0 mg/l. The primary response was obtained after 4 weeks; 0.25 mg/l IBA gave the best results, with a rooting percentage of 25%, mean number of 7.87 roots along with mean root length of 7.13 cm. However, all other concentrations of IBA failed to induce roots.

Key Words: *Lens culinaris*, lentils, rooting, *in vitro*, IBA

Mercimek (*Lens culinaris*)'te IBA'in *in vitro* Kök Gelişme Üzerine Etkisi

Özet: Mercimek *Leguminosaea* familyasına ait önemli bir bitki olup, *in vitro* köklenmesi oldukça zordur. MS besin ortamında gelişen Ali Dayı mercimek çeşidine ait 10 günlük sürgünler kesilerek, köklendirme için 0,25, 0,5, 1,0 ve 2,0 mg/l IBA içeren MS besin ortamına aktarılmıştır. Dört hafta sonra en yüksek köklenme oranı (%25), sürgün başına ortalama kök sayısı (7,87 adet) ve ortalama kök uzunluğu (7,18 cm) 0,25 mg/l IBA içeren MS besin ortamından elde edilmiştir. Ancak, diğer IBA uygulamalarında kök oluşumu uyarılamamıştır.

Anahtar Sözcükler: *Lens culinaris, mercimek*, köklenme, *in vitro*, IBA

Introduction

Lentil is an important crop of the family *Leguminosae*. Efforts are underway to study its rooting mechanism. Legumes are notoriously recalcitrant to tissue culture and are difficult to regenerate *in vitro*. Successful regeneration of legumes has been aided by species-specific determination of critical regeneration parameters such as explant source, genotype, media constituents (Parrot et al. 1992) and temperature. However, in lentil, the rooting of *in vitro* regenerated shoots present problems in achieving whole plant regeneration systems, and roots have been obtained sporadically in previous studies. We have observed that rooting responses are under the strict control of the genetic background of the starting material. However, all of the described procedures generally have yielded unsatisfactory frequencies of regeneration, and often involved extensive manipulation of culture conditions to obtain regenerants. Owing to lack of the availability of data on *in vitro* regeneration of lentil, an efficient method for plant regeneration in this species is necessary to extend the applications of tissue culture in lentils.

Efficient methods for inducing roots in lentils are equally important for whole plant regeneration, which could lead to developing morphologically normal plants after transformation.

Materials and Methods

Mature seeds of *L. culinaris* Medik. (2n=14) cultivar "Ali Dayı" were surface sterilized in 5% NaOCl for 20 minutes and thoroughly rinsed 5 times with sterile distilled water. The seeds were then germinated on MS basal medium containing 3% sucrose (w/v), 0.8% agar (w/v) in sterile Petri dishes (20 x 100 mm) sealed with stretch film for 6-10 days. The media were adjusted to pH 5.7 before autoclaving at 121°C for 20 minutes. Cultures were maintained in a growth chamber at

25±1°C under a 16 hour photoperiod provided by cool white fluorescent lamps (52 µmol m^{-2} s^{-1}). After 10 days, shoots (3-6 cm) were isolated and rooted on media containing indole 3 butyric acid (IBA) (0.25, 0.5, 1.0, and 2.0 mg/l). Each treatment was replicated 4 times and contained 4 plantlets in each magenta vessel. The primary response in this media was obtained after 4 weeks of culture. The main objective of this work was to develop an efficient rooting system. We studied the mean percentage of rooted shoots, mean number of roots, and mean length of roots per shoot. The rooted plants were transferred to pots containing vermiculite. For acclimatization, the plantlets were kept for seven days in plant growth chamber with 80-90% humidity under 25±1°C. All plants established well and developed a wide branched root system. Hardened plants were transplanted into soil mix and grown to maturity. Previously studied auxins such as NAA were not tested.

Results and Discussion

MSO medium containing 0.25 mg/l IBA resulted in 25% rooting, along with an average of 7.87 roots per shoot with a mean length of 7.13 mm (Table 1) all arising only after an initial callusing stage (Fig. 1a). Concentrations other than 0.25 mg/l IBA failed to induce rhizogenes (Fig. 1b). Rooted shoots could be transplanted after 2 weeks into commercial soil mixture and grown in pots to maturity under greenhouse conditions.

Table 1. Effect of different IBA concentrations on *in vitro* rooting of lentil.

IBA (mg/l)	Mean rooting percentage (%)	Mean number of roots	Mean root length/ shoot
0.5	0	0	0
1	0	0	0
2	0	0	0
0.25	25.0	7.87	7.13

The results suggest that further research is needed for improving root induction in lentils. In lentils (*Lens culinaris*), shoot regeneration by organogenesis or somatic embryogenesis has been reported by Williams

Figure 1. Effect of indole-3-butyric acid on root development in lentils. (a) Root development in a medium containing 0.25 mg/l IBA. (b) Inhibition of root development, but callus formation in a medium supplemented with higher IBA concentrations.

and McHughen (1986), Saxena and King (1987), Polanco et al. (1988), Singh and Raghuvansi (1989), and Malik and Saxena (1992) but with limited success in whole plant regeneration. In most of the studies, roots were only sporadically obtained from regenerated shoots. Williams and McHughen (1986) obtained a rooting efficiency of 11.0% when shoots were transferred to non- sterile sand. Polanco and Ruiz (1997) suggested a possible inhibitory effect of BAP on *in vitro* root formation in lentil. The common opinion is that BAP or cytokinins have inhibitory effects on rooting in legumes.

The frequency of whole plant establishment was relatively low in this study. However, the method presented here may be more feasable than others described earlier. Minor improvements in the method could lead to improved results for genetic manipulation of this important legume species.

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