http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (3): 998-1001, 1999 Research Article

Some Aspects of Adventitious Rooting in Microsperma Lentil CV-Masoor-85

Nafees Altaf, Javed Iqbal* and M. Salih Ahmad
Nuclear Institute for Agriculture and Biology, P.O.Box No.128, Jhang Road Faisalabad,
*Department of Botany University of Punjab, Lahore, Pakistan.

Abstract

Presence of auxins and high humidity in the initial stages of growth *in vitro* of 10-15 days old shoot cuttings is very essential for adventitious rooting in microsperma lentil in cultivar Masoor-85. The type and quantity of auxins is important. The basal segments of stem have more chances of rooting which gradually decreases for apical segments. Also age of shoots in culture has influence on rooting. Rooting is with normal polarity. Boron (30 mg/l) and MS (1/10 strength) salts in diluted concentration helps root induction and development. Moist peat and filter paper bridge both support rooting.

Introduction

Objective of this study was to optimize the conditions for the rooting of the *in vitro* regenerated shoots and explore the conditions influencing the establishment of plants in soil.

Roots are very important plant organs as they are involved in the acquisition of water and nutrients, anchorage of plants, synthesis of plant hormones and storage functions (Schiefelbein and Benfey, 1991). Profound perturbation of normal metabolism is required in order to regenerate the root system, to restore the thermodynamics of the whole plant and to sustain life (Haissig, 1985). However, rooting is genetically controlled (Altamura, 1996). This paper reports some of the factors studied for induction of adventitious rooting in cuttings of field grown Masoor-85 and also *in vitro* callus regenerated shoots.

Materials and Methods

Preparation of callus regenerated shoots: The callus was induced in dark from apices and cotyledonary nodes of germinating seedlings (3-4 day old) of Masoor-85 in MS + K (10mg/l) + GA (1mg/l) + 5% lentil seed extract. The calli were subcultured and maintained in the same medium in dark at 21 ± 1°C. Shoot regenerations were obtained in 16/8 hours light/dark cycle in the same culture medium. Shoot regenerations did not occur in darkness, exposure to light (4000) lux conditions was always necessary.

Effect of Auxins on adventitious rooting: Shoots regenerated from cotyledonary node callus were excised at the base and kept on moist filter paper in jars which contained 1/10 of MS salts (Murashige and Skoog, 1962) and IAA, IBA or NAA ranging from 0-5 - 2.0 mg/l. The percent shoots rooted were recorded after 21 days.

Formulation of rooting powder: The stem cuttings from the lower middle region of one month old plants of Masoor-85 were subjected to rooting. Rooting powder formulations are described in Table 2. The bases of the cuttings were dusted with rooting powder. High humidity was maintained for 10 days. Humidity was gradually reduced. Data was recorded after 10 days.

Effect of auxin on adventitious rooting of basal stem region: The basal stem sections of callus regenerated shoots from six weeks cultures were transferred to moist peat after treatments as described in Table 3. High humidity was maintained for 15 days. Humidity was gradually reduced and the data was recorded after 35 days.

Effect of boron, auxins, peatmoss, soil, filter paper bridge on rooting potential of basal stem segments: The basal portion of stem from 10 day old seedlings of Masoor-85 was transplanted for rooting with different treatments as mentioned in Table 4. The data was recorded after 15 days.

Rooting potential of stem sections in acropetal order: To probe the rooting potential of various stem segments, an experiment on six weeks old, around eight inch high Masoor-85 plants was conducted. The plants were cut above the cotyledons to remove the rooting system and then the shoots were cut into four equal parts. So from the base towards the apex, these were basal, lower middle, upper middle and upper portion. Twenty shoot sections of each type were inserted upright with normal polarity into peatmoss and twenty sections with inverse polarity. So overall, there were forty sections for each treatment. The stem base that was going into the peatmoss was dusted with rooting powder. Data was recorded after 15 days (Table 5).

Effect of age of *in vitro* regenerated shoots on adventitious rooting: The influence of age on the rooting potential of the regenerated shoots of one to five week old was considered. The control (seedling) shoots were cut from 0.5 cm above the cotyledons to remove the rooting system. The cut bases of callus regenerated shoots and seedling shoots were dusted with rooting powder and inserted in peatmoss. High humidity was maintained during first two weeks and then it was gradually decreased. The percent of shoots rooted after one month was recorded.

Results

The conditions were optimized for both the rooting of the *in vitro* regenerated shoots and control (seedling shoots) to explore the conditions influencing the establishment of plants in soil.

Effect of auxin on adventitious rooting: The results (Table 1) clearly indicated that the auxins have a positive role on adventitious rooting as rooting was induced by each type of auxins. (IAA, IBA, NAA) and at all concentrations (0.5 - 2mg/l) tried. However, IBA at 2mg/l had induced maximum 30 percent adventitious rooting. It clearly indicated that auxins enhanced rooting.

Table 1: Effect of auxin on adventitious rooting) in shoots cuttings derived from cotyledonary node (Masoor-85) callus

	Concentration mgs/litre			
Auxin	0.5	1.0	1.5	2.0
	% adventitious rooting			
NAA	5	8	9	18
IAA	3	7	11	15
IBA	7	10	28	30

Table 2: Rooting response of shoot cuttings of one month old seedlings after treatment with various rooting powders

Rooting powders	% shoots rooted	Root size
Talc + IBA (0.8%)	29.2 (24)	Medium size
Talc + MS salts	56.3 (23)	elongated
Talc + IBA (0.8%) + IAA (0.2%)	8.3 (23)	elongated
Talc + IBA (0.8%) + NAA (0.2%)	90.0 (22)	small size
Talc + IBA (0.8%) + IAA (0.2%) + NAA (0.2%)	62.5 (22)	small size
Talc + IBA (0.8%) - IAA (0.2%) + NAA (0.2%) + MS Salts.	33.3 (20)	medium/ small

Small roots = less than 0.5 cm long: medium roots, between 0.5 cm and 1 cm: elongated, more than 1 cm. Numbers in brackets refer to number of shoots used for each treatment.

Table 3: Effect of auxin on rooting of basal stem segments (six week old seedling) in peat.

Treatment	Rooting potential (%)
Only moisture	23*
MS (1/100 strength)	26*
Bases dusted with simple talc.	20*
Bases dusted with simple talc + NAA (0.2%) + IBA (0.8%).	39**

* % based on 100 cuttings. ** % based on 200 cuttings.

Formulation of rooting powder: Various rooting powders were formulated as mentioned in Table 2. It seemed that the root initials could form even without auxins as sometimes root primordial structures were visible although no histological study was obtained made. Auxins enhanced rooting process as maximum rooting (90%) was with IBA (0.8%) + NAA (0.2%) in simple talc. The rooting process was also speeded by high humidity and vigour of shoots rooting.

Table 4: Rooting potential (recorded after 15 day) of basal segments of stem (10 day old seedlings) in different auxins and boron of Masoor-85.

Treatment	Rooting Response
Tap Water, P.	+ +
H_3BO_3(30 mg/l)^{xx}, P.	+ + +
B^{xx} + RP, P.	+ + + +
RP^{xxx} (FPB)	+ + + + + +
RP + Soil.	+ + +
IAA (MS^x), P.	+
IBA (MS^x), P.	+ +
NAA (MS^x), P.	+ +
MS^x, P.	+
RP (MS^x), P.	+ + + +

IAA, IBA, NAA (each 1 u M); MS^x (1/10th strength). FPB, Filter paper bridge. RP^{xxx} (simple talc + NAA 0.2% + IBA 0.8%) P, Peatmoss; + >10%, + + 10-15%, + + + 15-20%, + + + + 20-25%, + + + + + 30-35%.

Effect of auxin on adventitious rooting of basal stem region: The untreated and treated basal stem segments (Table-3) were inserted into peat plugs. Humidity was essential. From the experiment it was concluded that stem base had inherent ability of root primordia formation which slightly increased with dilute salts. However auxins NAA, and IBA, enhanced the rooting potential.

Effect of boron, auxins, peatmoss, soil, filter paper bridge on rooting potential of basal stem segments: The rooting powder was again tested with various supporting combinations (Table 4). Filter paper bridge coupled with rooting powder (combination 4) was a better system for root induction as compared to the peat and soil (combination 5). Boron had positive response for rooting (combination 3). The MS salts have also gave positive response.

Effect of time period in culture after shoot regeneration on adventitious rooting: The regenerated shoots after weekly intervals were studied for rooting to test if the long stay in culture vessel had any positive or negative effect on rooting. The time of shoot regeneration was weekly compared with the same age of shoots from control seedlings. It was found that the optimum rooting (21%) took place after two weeks of shoot regeneration. However, the rooting (59%) in the seedlings was optimum after three weeks age. The regenerated shoots had root primordia with some visible roots. Longer keeping of shoots in agar medium and the elongated shoots with thin stem were making difficult for further survival as rooted plants (Table 5).

Rooting potential of upper and lower portions of stem: All the four segments mentioned as basal, lower middle, upper middle and upper portion both with normal and reverse polarity were assessed for rooting. In microsperma lentil, there were no rooting with reverse polarity. The basal portion nearer to the cotyledon region had the maximum rooting response (45%) and it decreased to 25, 10, 0 per cent with the distance from the basal portion (Table 6).

Table 5: Rooting* of shoot cuttings after 1-5 week of regeneration in light from callus** induced in dark.

Age of shoot	Percent rooted shoots	
	Regenerated	Seedlings shoots control)***
1 week	14	28
2 week	21	53
3 week	16	59
4 week	13	37
5 week	9	22

*bases dusted with rooting powder and shoot base inserted in peat moss. **Callus medium: MS+K (10mg/l) +GA (1mg/l). ***Control is the seedling shoot cuttings, 0.5cm above the cotyledons.

Table 6: Effect of stem portion and polarity on per cent** rooting in Masoor-85 (six week old).

Stem section*	Normal polarity	Reverse polarity
Basal	45	0
Lower middle	25	0
Upper middle	10	0
Upper	0	0

x Bases dusted with rooting powder. Peatmoss was used for growth. xx % based on 20 cuttings.

Discussions

The major hormones discovered to date fall into six classes, auxins, cytokinins, gibberellins, abscisic acid, ethylene and oligosaccharins. However, the mode of action of growth regulators in organogenesis is little understood. The balance of auxins and cytokinins in a growth medium is generally thought to determine the type of organ produced (Skoog and Miller, 1957). Auxins and cytokinins appear to be the major endogenous factors regulating adventitious root formation. The cytokinins are considered inhibitory (Bollmark and Eliasson, 1990), whereas the auxins are stimulatory (Gasper and Hofinger 1988). Although IAA is the major auxin, the probability that the other natural auxins like IBA, PAA, 4CT-IAA and IPA (Epstein *et al.*, 1989) participate in regulation can not be discounted. In soybean kinetin 2.0 mg/l and IBA 2.0 mg/l developed roots (Setta and Kumari, 1998). However, in our work of lentil, Kinetin induced buds and IBA induced rooting. Jarvis and Shaheed (1986) found that there was no correlation between the number of roots regenerated and the total amount of auxin accumulated in the hypocotyl. Haissig (1970) with brittle yellow cuttings stated that IAA can not produce root primordia unless competent cells are present and that the formation of these cells was not induced by IAA. Microsperma needed auxins for root induction in the regenerated shoots. The basal stem sections of Masoor-85 had tendency to form roots but rooting was enhanced by auxins. The rooting potential decreased as culturing moved from the base to the apical portion.

Addition of boron had a positive effect on rooting. The filter paper bridge and the moist peat proved better. High humidity (90-100%) was very essential around the shoot during rooting and weaning period. Wilting was because of many reasons as weak stems, succulent or translucent stem, absence of roots irregular rooting or weak roots as they easily detach from stems associated with callusing at the base. Tissue culture plants were either poor in chlorophyll content or enzyme responsible for photosynthesis were inactive or absent altogether (Dhawan and Bhojwani, 1987). Poor mesophyll differentiation and weak vasculature of the leaves made plants highly susceptible to transplantation (Donnelly and Vidaver, 1984). In this study *ex-vitro* rooting was tried for obvious reasons of economy and the quality of roots.

In microsperma lentil, the losses during weaning stage depended not only on the culture itself but also on the field environment like the season, day and night temperature and humidity. The surviving lentil plants were grown to maturity where they set pods. The indication of survival is by growth of new leaves. Tissue culture leaves never become normal and it is imperative that new leaves develop quickly to recover plant growth.

Also adventitious rooting was influenced by time period shoots were kept in culture or in other words, the age of the regenerated shoots was important in Masoor-85. Keeping for longer time in cultures made the survival of shoots difficult during the root induction period. The age of the shoots to be rooted is very important as the basal shoots from the plants near to flowering or in reproductive phase are extremely difficult to root in lentil. On the contrary, shoots from young plants in vegetative phase can induce rooting with the help of auxins and in humid conditions. Rooting is a very complex phenomenon in which several factors play a role.

References

Altamura, M.M., 1996. Root histogenesis in harbacious woody explant cultured *in vitro*. A critical review. Agronomia (Paris), 16:589-602.

Bollmark, M. and L. Eliasson, 1990. Ethylene accelerates the breakdown of cytokinins and thereby stimulates rooting in Norway spruce hypocotyl cuttings. Physiol. Plantarum, 80: 534-540.

Dhawan, V. and S.S. Bhojwani, 1987. Hardening in vitro and morphophysiological changes in the leaves during acclimatization of micropropagated plant of *Leucas tencocephala* (Lam.) de Wit. Plant Sci., 53: 65-71.

Donnelly, D.J. and W.E. Bidaver, 1984. Leaf anatomy of red raspberry transferred from culture to soil. J. Am. Soc. Hortic. Sci., 109: 172-176.

Epstein, E., Chen, J.D. K-H and Cohen, 1989. Identification of indole-3-butyric acid as an endogenous constituent of maize kernel and leaves. Plant Growth Regulation, 8: 215-223.

Gasper, T. and M. Hofinger, 1988. Auxin metabolism during adventitious root formation. In TD Davis, B.E. Haissig, N. Sankhla eds., Adventitious root formation in cuttings. Advances in Plant Sciences Series, Vol. 2. Dioscorides Press, Port land, OR, pp: 117-131.

Haissig, B. E., 1970. Influence of Indole-3 acetic acid on adventitious root primordia of brittle willow. Planta, 95: 27-35.

Haissig, B.E., 1985. Metabolic processes in adventitious rooting. In MB Jackson, ed. New Root Formation in Plants and Cuttings. Martinus Nijholf/Dr.W.Junk Publ. The Hague, pp: 141- 190.

Jarvis, B C. and Shaheed, A.I. 1986 Auxin uptake, transport and accumulation in relation to rooting and aging of mungbean cuttings. Biochem. physiol. Pflanzen, 181: 597-604.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures Physiologia. Plant, 15: 473-496.

Schiefelbein, J. W. and P.N. Benfey, 1991. The development of plant roots: New approaches to underground problems. The Plant Cell, 3: 1147-1154.

Setta, A. and B.D.R. Kumari, 1998. Plant regeneration from cotyledonary explants of soybean. Advances in Plant Sci., 11:193-197.

Skoog, F. and C.O. Miller, 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Exp. Biol., 11:118-131.