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S.Ü. ZİRAAT FAKÜLTESİ DERGİSİ
YAYIN İLKELERİ

1- S.Ü. Ziraat Fakültesi Dergisi'nde öncelik sırasıyla mesleki ve teknik konulardaki orijinal araştırma, derleme yazıları yayınlanır. Ancak, bir dergideki derleme makalesi sayısı en çok iki adet olmalıdır.

2- Dergiye sunulan yazılar, makale konusu ile ilgili uzmanlık dalındaki bir danışmana gönderilir. Danışman görüşleri yayın komisyonunda değerlendirildikten sonra yayını konusunda karar verilir.

3- Eserin başlığı metne uygun, kısa ve açık olmalı ve büyük harfle yazılmalıdır.

4- Orijinal araştırmaların yazılış tertibi aşağıdaki şekilde olmalıdır !

a- Eserin yazar veya yazarlarının adı tam olarak küçük harflerle, başlığın alt ortasına yazılmalı ve ayrıca yazar veya yazarların ünvan, çalıştıkları yer isim veya isimlerin sonuna konacak dipnot (*, **) işaretleriyle ilk sayfanın altına bir çizgi çizilerek metinden ayrı bir şekilde belirtilmelidir. Varsa araştırmayı destekleyen kurumların ismi de bu dipnot içinde belirtilmelidir.

b- Eserin bölümleri şu sıraya uygun olmalıdır : Türkçe ve yabancı dilde (ingilizce, almanca ve fransızca) Özet, Giriş, Materyal ve Metod, Araştırma Sonuçları ve Tartışma, Kaynaklar. Her bölüme ait başlık satır hizasında koyu bir şekilde yazılmalıdır.

c- Türkçe ve yabancı dilde verilen özetlerin herbiri 200 kelimeyi geçmeyecek şekilde hazırlanmalı ve yabancı dilde özetin başına eserin başlığı aynı dilde,ve büyük harflerle yazılmalıdır. Türkçe özetin altına anahtar kelimeler, İngilizce özetin altına key words yazılmalıdır.

d- Metin içerisinde kaynaklardan yararlanırken (Soyadı, sene) sistemi kullanılmalıdır. Örnekler : - Black (1960) .... olduğunu tespit etmiştir.

- Bitkilerin fotoperyoda gösterdikleri reaksiyon bazı kimseler tarafından araştırılmıştır (Weaver, 1933; Galston, 1961 ve Anderson, 1968).

- Eser üç veya daha fazla kimse tarafından yazılmışsa ilk yazarın soyadı ile örneğin "Anderson ve ark. (1945) şeklinde yazılmalıdır. Yararlanılan kaynağın yazarı veya yayınlayan kurum bilinmeyen yazar ismi yerine "Anonymous" yazılmalıdır.

e- Kaynak Listesinin Hazırlanması : Kaynak listesi yazarların veya ilk yazarların soyadlarına göre alfabetik olarak sıralanmalıdır. Kaynak listesinde eseri yazan yazarların hepsinin isminin verilmesi gerekir. Örnek; - Kacar, B., 1972. Eserin adı "A.Ü. Ziraat Fak. Yayınları : 453, Uygulama klavuzu : 155, 450-455, Ankara.

- Snedecor, G., Hanway, A.H., Hoane, H.G. ve Andecor, G.H., 1961. "Eserin adı" Agron. Jour. 7 (2) : 311-316.

5- Gönderilecek yazılar, Şekil ve Tablo dahil olmak üzere 15 daktilo sayfasını geçmeyecek şekilde hazırlanmalıdır.

6- Eserde verilecek Tablo, Çizelge ve Cetvel'in tamamı dergide birlik sağlamak açısından "Tablo" olarak isimlendirilmeli ve numaralandırılmalıdır. Ayrıca Tablo numara ve ismi örneğin "Tabro 1. Toprakların ..." şeklinde tabloların üst kısmına yazılmalıdır. Tablolar başka kaynaktan alınmışsa açıklamasından hemen sonra kaynak gösterilmelidir (Örneğin, "Black, 1961" gibi).

7- Şekil ve Grafikler aydınger kağıdına çini mürekkebi ile çizilmeli, resimler parlak fotoğraf kartına siyah beyaz ve net basılmış olmalıdır. Eserlerde kullanılan grafik ve fotoğraflarda "ŞEKİL" olarak isimlendirilip numaralandırılmalı ve şekil altına (Örneğin, Şekil 1. Traktörlerde ..." gibi) açıklamaları yazılmalıdır. 13x18 cm'den daha büyük şekil kabul edilmez.

8- Yazar veya yazarlar eserlerini gönderirken, başka bir yerde yayınlanmadığını veya yayınlanmak üzere verilmediğini yazılı olarak belirtmelidirler.

9- Yazıların sorumlulukları yazarlarına aittir.

10- Eserin basımı sırasındaki düzeltmeler yazarınca yapılır. Eserlere telif ücreti ödenmez.

11- Sürekli yazılar yayınlanmaz.

12- Derginin bir sayısında ilk isim olarak bir yazarın üçten fazla eseri basılmaz.

13- Yayınlanmayan yazılar iade edilmez.

YAYIN KOMİSYONU

İÇİNDEKİLER

(CONTENTS) *Sayfa No :*

S.Ü. Ziraat Fakültesi Dergisi 11(14) : 1-13, 1997

YEMEKLİK DANE BAKLAGİLLERDE DOKU KÜLTÜRÜ VE BİTKİ REGENERASYONU ÇALIŞMALARI

Mehmet BABAOĞLU*

ÖZET

Yemeklik dane baklagillerde türler ve cinsler arası uyuşmazlıklar mevcut gen havuzunu sınırlamaktadır. Bu nedenle genetik manipulasyon ve *in vitro* teknikleri konvansiyonel ıslaçıya yeni varyasyonlar ortaya koymak için büyük öneme sahiptir. Ayrıyca, bu tür bir yaklaşım yine ıslahçıya süre bakımından büyük kazançlar sağlayacaktır.

Yemeklik dane baklagiller, manipüle edilmesi ve iyileştirilmesi gereken bir çok karakterleri olmasına rağmen *in vitro* tekniklerine gösterdikleri tepki bakımından oldukça büyük zorluklar arz etmektedirler. Fakat istenmeyen karakterlerin azaltılması ve çeşitli dayanıklılık genlerinin ilgili genotipe aktarılabilmesi için tekrar edilebilir bir bitki regenerasyon metoduna ihtiyaç vardır.

Bu çalışmada yemeklik dane baklagillerin genetik manipülasyonu üzerine yapılan çalışmaların en önemli köşe taşlarını oluşturan araştırmalar tablo halinde verilmiştir. Burada amaç, yemeklik dane baklagillerde çalışmaya başlamış veya yeni başlayacak olan araştırmacılara yol göstermek ve özellikle belirtilen cins, tür veya varyetelerde *in vitro* tekniklerinde kullanılması gereken en uygun bitki kısımları, besin ortamları ve bitki büyüme regülatörleri kombinasyonları konularında rehberlik etmektir.

Anahtar Kelimeler : Kallus, mikropropagasyon, apikal meristem, köklendirme.

ABSTRACT

TISSUE CULTURE AND PLANT REGENERATION IN GRAIN LEGUMES : A REVİEW

The available gene pool in grain legumes is restricted by the sexual incompatibility of many interspecific and intergeneric crosses. Genetic manipulation and *in vitro* cultures provide substantial advantages in broadening the genetic variability and reducing the time required for introgression of new traits.

Grain legumes are generally recalcitrant crops with respect to their *in vitro* responses and require manipulation for undesirable characters they possess.

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Similarly, genetic transformation of any species necessitates a reproducible plant regeneration system.

This study was intended to give an initial guidance, to researchers on grain legumes, about the most appropriate explant, medium and plant growth regulator combination for a given species. All previous works were summarised in table.

Key Words : Callus, micropropagation, apical meristem, rooting.

INTRODUCTION

The grain legumes, also called pulses, currently of importance to World Agriculture include; pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medic.), common bean (*Phaseolus vulgaris* L.), Mung bean (*Vigna radiata* L.), cowpea (*Vigna unguiculata* L.), pigeon pea (*Cajanus cajan* L.), lupin (*Lupinus* species), soybean (*Glycine max* L.) and groundnut (*Arachis hypogea* L.). The latter two, groundnut in particular, are also grouped with the oil crops (Williams, 1986).

Legumes are the only plants which do not depend entirely on external applications of nitrogenous fertilisers for their nitrogen supply. They can sometimes supply up to 50% of their nitrogen requirement from ammonia produced after fixation of nitrogen from the air as a result of the symbiotic relationship with the gram-negative soil bacterium, *Rhizobium* (Chrispeels and Sadava, 1994).

Grain legumes should be supported by agricultural policies as well as improvements in yield and quality, since according to the GATT (General Agreement on Tariffs and Trade) report, there is no area limitation for the cultivation of grain legumes as there is for oil crops and cereals (Delplancke, 1993). The EU (European Union) Common Agricultural Policy, from 1978 onwards, has been to encourage the cultivation of protein crops, especially grain legumes, for animal feed to make up the deficiency in protein-rich material supply (Muel, 1995). As a result of this policy, production of grain legume crops has increased, providing 19% of the total 36% of protein production in the EU (Ranalli, 1995).

Grain legumes, in general, are recalcitrant crops *in vitro* and currently require the establishment of reproducible *in vitro* protocols for their genetic improvement (Sator, 1990).

CALLUS AND SUSPENSION CULTURES IN GRAIN LEGUMES

A high auxin:cytokinin ratio usually induces callus formation from *in vitro* cultured explants. The auxin 2,4-D is usually sufficient when incorporated in the culture medium for the induction of callus in monocotyledons, however, for dicotyledonous species, a cytokinin is almost always present as well (George, 1993).

Callus induction in the legume genus, *Pisum*, is achieved more readily than for other grain legume species, since most pea explants (shoot tips, hypocotyls, stems, leaves) give callus followed by shoots or roots (Christou, 1992). MS basal medium is the most appropriate culture medium for callus induction often incorporating high levels of Picloram, NAA or 2,4-D with low levels of BAP or KIN. Picloram and 2,4-D have also been successfully used with *Phaseolus* tissue cultures, with the addition to the culture medium, of coconut water. NAA has been less effective at similar concentrations while IAA was ineffective. Limited organogenesis from callus cultures is also possible (Christou, 1992).

Cell suspension cultures can be established for many grain legumes (Christou, 1992), whereby explant sources (immature embryos or cotyledons) in media containing 2,4-D give rise to embryogenic cell suspensions as in for example, soybean [*Glycine max*; Finer (1994)].

PLANT REGENERATION AND MICROPROPAGATION IN GRAIN LEGUMES

A summary review of regeneration status of the major grain legumes is presented in Table 1. In most plant regeneration and micropropagation studies, the use of MS medium with BAP (0.2-10.0 mg l^{-1}) either alone or in combination with NAA was common. Plant cells also are able to use reduced nitrogen from the ammonium ion and from amino acids (e.g. L-glutamine, L-proline) (George, 1993). Franklin *et al.* (1991) found that L-glutamine (1461 mg l^{-1}) successfully replaced inorganic nitrogen source in culture media for cotyledonary node explants of *Phaseolus vulgaris*. The availability of nitrogen and the form in which it is presented markedly influences the growth and morphogenesis of plant and cell cultures. Changes in the NO_3^-/NH_4^+ ratio enabled direct and indirect shoot formation, embryogenesis and axillary shoot proliferation in a wide range of tissue cultures (George, 1993). Nadolska-Orcyzk (1992) reported somatic embryogenesis from immature cotyledons of *Lupinus* species in MS medium with a lowered NH_4NO_3 concentration (Table 1).

Recently, a different approach was developed for *in vitro* regeneration and propagation of some major grain legumes. In their rapid and least labour-requiring protocols, Malik and Saxena (1992b, c) achieved plant regeneration using mature seeds, from a number of grain legumes germinated directly on MS medium containing TDZ or BAP (Table 1).

Most regeneration systems in grain legumes rely on the use of tissues carrying pre-existing meristems. Nodal explants including cotyledonary nodes (*Glycine max*, Cheng *et al.*, 1980; *Phaseolus vulgaris*, Franklin *et al.*, 1991; *Pisum sativum*, Nauerby *et al.*, 1991; *Cicer arietinum*, Brandt and Hess, 1993), shoot apical meristems or shoot tips (*Phaseolus vulgaris*, Kartha *et al.*, 1981; *Lens culinaris*, Williams and McHughen, 1986; *Glycine max*, McCabe *et al.*, 1988) were the most

Table 1. Studies Relating to *In Vitro* Regeneration of Grain Legumes : a Summary.

Plant Species	Explant specification	Media (all PGRs[a] in mg.l^{-1})	Response	Notes	References
Cicer arietinum cv. C-235	Shoot tips and nodal segments (1.0 cm) of seedlings grown on 1/2 MS medium for 9 d.	MS[a] plus 0.00186 NAA and 0.225-2.25 BAP for induction, 0.00186 NAA and 0.225 BAP for shoot multiplication.	Five shoots per explant in 8 w, 9.6 nodal segments with shoots per explant after 4 w. 0.2 IBA effective for rooting.	One axillary shoot from each nodal segment. Rooting was significantly influenced by the presence of vitamins.	Barna and Wakhlu, 1995.
Cicer arietinum cvs. Annigeri and ICCV6.	Cotyledonary nodes. Meristem tips.	B5[a] medium with 1.0 BAP and DKW-C-a medium with 1.0 BAP and 0.01 IBA. WH medium with 0.5 IBA for rooting.	Seven shoots from each node. Multiple shoot induction (96 %), with 10 shoot per meristem after 4-6 w.	Meristem tips produced more shoots than cotyledonary nodes. Vitrification of shoots during rooting.	Brandt and Hess, 1993.
Cicer arietinum L.	Shoot apical meristem with subjacent tissues from 4-6 d old seedlings	MS with B5 vitamins and 3 % (w/v) sucrose with 0.0-2.25 BAP and 0.0-0.86 NAA.	2.25 BAP alone can induce multiple shoot formation, and 0.25 BAP maintains shoot development.	Axillary shoots can only be rooted when 0.2 IBA is used. Rooting is auxin specific.	Kartha *et al.*, 1981
Cicer arietinum PGI, PG12 and PG5 genotypes.	Mature embryo axes.	MS liquid medium with 3 % (w/v) sucrose and 3.0 2, 4, 5-T.	Callus and subsequent somatic embryo formation leading to recovery of plants.	Somatic embryos originate directly from epidermal and subepidermal cells.	Sagara *et al.*, 1995
Glycine argyrea Tind. various accessions.	Cotyledons, leaflets and petioles.	B5 medium with 3 % (w/v) sucrose plus i) 1.1 BAP, 0.005 IBA, ii) 1/2 B5, no hormones.	i) Callus induction (2 w), ii) Shoot regeneration from callus after 3-6 subcultures.	Phenolic production reduced by rapid subcultures.	Hammatt *et al.*, 1989.

[a]PGR : Plant growth regulator, CPPU : *N*-(2-Chloro-4-pyridyl)-*N*' -phenylurea, DHZ : DL-Dihydrozeatin, TDZ : thidiazuron; 1-phenyl-3-(1, 2, 3-thiadiazol-5-yl) urea, B5 medium : Gamborg *et al.*, 1968, LS medium : Linsmeier and Skoog, 1965, MS medium : Murashige and Skoog, 1962, WH medium : White, 1965.

Table 1 continued.

Plant Species	Explant specification	Media (all PGRs in $mg.l^{-1}$)	Response	Notes	References
Glycine max (L.) Merr.	Embryonic axes with exposed apical meristem devoid of primary leaves.	MS with 3 % (w/v) sucrose, 0.037 NAA and 3.0 BAP plus thiamine and proline for 2 w then on MS with 0.04 IBA and 0.3 BAP	Multiple shoots from both primary and axillary meristems under photoperiod (16 h).	Rooting on hormone-free medium or grafting of shoots onto 10 d old seedlings.	McCabe *et al.*, 1989.
Glycine max (L.) Merr.	Immature shoot tips without primary leaves.	MS with 0.037 NAA and 3.0 BAP plus thiamine, proline for 2 w then on MS with 0.38 BAP.	Multiple shoots proliferation from shoot tips and axillary meristems. Rooting in B5 without hormones.	Layers in addition to L1, L2 in the apical meristem, also involved with shoot formation.	Sato *et al.*, 1993.
Glycine max (L.) Merr. cv. Mandarin.	Shoot apical meristems with subjacent tissues and two leaf primordia.	MS with B5 vitamins and 3 % (w/v) sucrose i) 0.186 NAA, 0.225 BAP, ii) 0.186 NAA, 0.0225 BAP	i) Whole plant regeneration (33 %, no multiple buds, ii) Multiple buds but no plant regeneration.	Formation of multiple buds prevented whole plant regeneration.	Kartha *et al.*, 1981.
Glycine max (L.) Merr. cvs. Williams, Amsoy and Dare	Cotyledonary segments from BAP pre-treated seedlings.	Modified B5 with 3 % (w/v) sucrose plus 0.005 IBA and 0.045-4.5 BAP	Two axillary shoots from each node, BAP (>2.25) inhibits main shoot development.	BAP reduces apical dominance. Rooting of regenerated shoots difficult.	Cheng et al., 1980.
Lens culinaris Medik.	Portions of shoot meristem.	MS with 3 % (w/v) sucrose, 10.0 KIN and 0.1 GA3 for 4 w in the dark then, transfer to 16 h photoperiod.	Callus formation in the first step, multiple shoot formation from the callus (50 %) in the second step.	Only callus from shoot meristem produces multiple shoots. Rooting not successful.	Williams and McHughen, 1986.

Table 1 continued.

Plant Species	Explant specification	Media (all PGRs in $mg.l^{-1}$)	Response	Notes	References
L. angustifolius cv. Turkus.	Callus derived from hypocotyl explants.	LS (modified) plus 0.1 NAA and 1.0 BAP (S4). S4 plus 0.06 NAA and 0.6 BAP (S6) for shoot elongation. S4 plus 0.6 NAA for rooting.	Multiple shoot induction from callus cultures. Hypocotyl-derived callus was nost responsive (90-100 %).	Type of cytokinin did not effect regeneration capacity of explants. NAA effective for root induction.	Sroga, 1987.
L. angustifolius cvs. Emir, Mirela, *L. mutabilis* line P, L. *albus* cv. Bac.	Immature cotyledons excised from pods (16-26 d post-flowering)	B5 supplemented with 5.0 2,4-D alone or with 0.25 KIN for induction, MS with lower NH_4NO_3 plus 0.1 ABA, 0.1 BAP for somatic embryo germination	Somatic embryo formation from submarginal tissues of cotyledons. 2,4-D was more effective than NAA for induction.	Embriyo formation and development required an appropriate sequence of media.	Nadolska-Orczyk, 1992
L. luteus cv. Aurea	Hypocotyl segments.	Modified Téoulé & Datteé (1986) medium with 0.02 NAA and 2.0 2,i-p for shoot regeneration	Plant regeneration via shoot organogenesis. Callus formation inhibited shoot regeneration.	Agar concentration not less than 1.0 % (w/v) for successful shoot regeneration.	Daza and Chamber, 1993.
L. texensis	Cotyledonary nodes containing hypocotyl tissue.	MS with 1.0 BAP (3-4 w) for shoot induction, MS with 5.0 NAA for rooting of shoots.	Shoot formation after 4 w. BAP was more effective than KIN. Rooting of shoots was low (14 %).	IAA and IBA had no effect on rooting. Rooting was the main problem.	Upadhyaya *et al.*, 1992
Phaseolus acutifolius, *P. aureus*, *P. coccineus*, *P. wrightii*.	Seeds germinated and grown on medium with BAP.	MS with B5 vitamins, 3 % (w/v) sucrose solidified with 0.25 % (w/v) Phytagel plus 18.0 BAP.	Shot regeneration and somatic embryogenesis.	No auxin necessary for induction of somatic embryos.	Malik and Saxena, 1992 a.

Table 1 continued.

Plant Species	Explant specification	Media (all PGRs in $mg.l^{-1}$)	Response	Notes	References
Phaseolus vulgaris cv. Dark Red Kidney	A cotyledon and a small portion of embryonic axis within 1.0 mm of epicotyl and hypocotyl.	Seeds germinated on MS with 1.125 BAP. Explant culture on MS devoid of inorganic nitrogen, substituted by L-glutamine plus 3.37 BAP, 0.7 GA_3.	Buds and shoots were produced from each explant.	Buds and shoots developed from peripheral layers of meristematic rign.	Franklin *et al.*, 1991.
Phaseolus vulgaris cv. Dwarf Green Stringless	Shoot apical meristems with subjacent tissues.	MS with B5 vitamins and 3 % (w/v) sucrose, plus 2.25 BAP	2.25 BAP induced multiple buds, rooting occurred after 2 weeks.	High BAP alone induces multiple bud formation	Kartha *et al.*, 1981.
Phaseolus vulgaris L.	Nodal explants.	MS with B5 vitamins, 3 % (w/v) sucrose, 1.1 BAP or 0.062 CPPU[a] or 0.055 TDZ.	CPPU and TDZ supplemented media resulted in more shoots.	Axillary shoot regeneration was enhanced by dark incubation and BAP pre-conditioning.	Mohamed *et al.*, 1992.
Phaseolus vulgaris various cultivars.	Intact seedlings grown in the presence of plant growth regulators	MS with B5 vitamins, 3 % (w/v) sucrose solidified with 0.25 % (w/v) Phytagel plus 2.2 TDZ[a] or 18.0 BAP.	Shoot regeneration from axillary buds, originating from subepidermal tissues.	Direct shoot regeneration from intact seedlings with short exposure to TDZ (7d).	Malik and Saxena, 1992 b.
Pisum sattvum cvs. Century, Afghanistan, Laxtons, Progress.	Apical domes devoid of leaf primordia (200-300 µm in length)	B5 plus 0.186 NAA and 0.11 BAP for induction, 1/2 B5 with 0.186 NAA for rooting.	Callus formation and shoot initiation.	NAA only, induced rooting, with cytokinins and high mineral salts inhibitory.	Kartha *et al.*, 1974.

Table 1 continued

Plant Species	Explant specification	Media (all PGRs in mg.l^{-1})	Response	Notes	References
Pisum sativum L. genotype PI244253	Axillary buds of cotyledonary nodes withut cotyledons	MS medium with 1.0 BAP, 1/2 B5 medium without PGR's for rooting of regenerated shoots.	Rapid shoot regeneration with minimum callusing.	Shoot regeneration is independent of genotype.	Jeckson and Hobbs, 1990.
Pisum sativum L. *Cicer arietinum* L. *Lens culinaris* Medik	Mature seeds.	MS with B5 vitamins, solidified with 0.25 % (w/v) Phytagel plus 2.2-6.0 TDZ and some other cytokinins. Rooting on MS with 0.46 NAA (40-50 %).	Maximal shoot regeneration from adjacent basal parts of epicotly with 4 d exposure to 2.2 TDZ. Over-exposure to TDZ (> 3 d) inhibits roting.	Chickpea and lentil produced fewer shoots than pea. High TDZ inhibited secondary root formation or callus in chickpea.	Malik and Saxena, 1992 c.
Pisum sativum L. cvs. Orb and Consort.	Immature cotyledons.	MS with 0.5 BAP, 4.0 NAA for shoot regeneration, 5.0-10 NAA for somatic embryogenesis.	Somatic embryos and shoot organogenesis.	Explant orientation was important. Varying genotypic responses.	Özcan *et al.*, 1993.
Pisum sativum L. various cultivars.	Shoot nodal explants, shoot apices.	MS with B5 vitamins plus 1.0 2,4-D or 1.0 Picloram for induction.	Somatic embryos. Picloram and 2,4-D required for induction.	Genotype and explant source dependent.	Van Dorne *et al.*, 1995
Pisum sativum various cultivars.	Nodal thin cell layers.	B5 liquid medium with glucose, DHZ[a] and IBA	Regeneration and multiplication of shoots from axillary buds.	Presence of IBA results in more shoot regeneration.	Nauerby *et al.*, 1991.

Table 1 continued.

Plant Species	Explant specification	Media (all PGRs in $mg.l^{-1}$)	Response	Notes	References
Vicia narbonensis	Excised shoot tips	MS medium with; 1.0-10 Picloram. MS medium with 1.0 NAA. MS medium with cytokinins	Callus enduction Somatic embryo induction. Embryo germination.	Somatic embryo induction via a callus stage and maturation of embryos in another medium.	Pickardt *et al.*, 1991.
Vigna ungiuculata (L.) Walp. cv. Vita 5-Exita	Shoot apical meristems with subjacent tissues.	MS with B5 vitamins and 3 % (w/v) sucrose plus 0.0-0.125 BAP and 0.0-0.093 NAA.	Whole plant regeneration (100 %) on PGR-free medium and use of PGR reduced regeneration frequency.	Endogenous hormones are sufficient for stimulation of regeneration.	Kartha *et al.*, 1981.

frequently used explants for the induction of multiple buds and/or shoots, which usually originated from subepidermal cells (Table 1).

Apical meristems have been the focus of many culture-based studies and plant development in general. Despite minor differences, the organisation and functioning of all shoot apical meristems is essentially the same. The terms "shoot apex", "shoot apical meristem" and "shoot tips" are often used synonymously or as discrete terms depending upon authors (Medford, 1992).

Studies relating to the use of shoot apical explants of grain legumes for the induction of morphogenesis (multiple buds or shoots) can be placed in the following categories: i) excision and culture of apical meristems devoid of leaf primordia [(*Lupinus* species; Ball, 1946), *Pisum* species (Kartha *et al.*, 1974), *Lens* species (Williams and McHughen, 1986)], ii) exposure of apical meristems which are still attached to the subjacent tissues [*Cicer* species, *Glycine* species (Kartha *et al.*, 1981)], iii) exposure of apical meristems (domes) from which primary leaf primordia were removed [*Glycine* species (McCabe *et al.*, 1988)], iv) whole shoot tips as explants [*Cicer* species (Brandt and Hess, 1993)] and v) apical meristems devoid of initial apical layer(s) [*Lupinus mutabilis* Sweet (Babaoglu, 1996)].

In general, *in vitro* -generated vegetative cuttings from grain legumes are more successfully rooted than shoots recovered from cells/tissues *in vitro*. Rooting of regenerated shoots can be induced by NAA or IAA but is still a low frequency event and hence problematical (Christou, 1992). Root formation is generally inhibited by cytokinins (George, 1993), for example, BAP and TDZ inhibit root formation in regenerated shoots of *Pisum* species and Phaseolus species (Kartha *et al.*, 1974; Malik and Saxena, 1992c respectively).

Rhizobial lipo-oligosaccharides (nod factors) have been shown to induce root hair initiation in *Vicia* species (Spaink, 1992) and could be helpful to stimulate rhizogenesis (Prof. E.C. Cocking, pers. comm.) in recalcitrant species. Grafting of regenerated shoots was found to overcome the rooting problem in some legumes [*Glycine max* (McCabe *et al.*, 1988), *Pisum sativum* (Tegeder *et al.*, 1995), *Vicia faba* (Böhmer *et al.*, 1995)], whilst *Agrobacterium rhizogenes* has been used to root *in vitro* some difficult-to-root *Lupinus* species (Babaoglu, 1996).

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