韓國林學會誌 84(2) : 145 - 150, 1995
Jour. Korean For. Soc. 84(2) : 145 - 150, 1995

Plant Regeneration from Callus Cultures of Black Locust(*Robinia pseudoacacia* L.)[1]

Jong Ho Woo[2], Myung Suk Choi[3] and Young Goo Park[3]

아까시나무(*Robinia pseudoacacia* L.)의 callus배양에 의한 식물체 재분화[1]

우종호[2] · 최명석[3] · 박용구[3]

ABSTRACT

A plant regeneration system using shoot basal callus of *in vitro* cultured black locust(*Robinia pseudoacacia* L.) was established. Shoot basal callus was induced on MS medium supplemented with BA or NAA, and were more proliferated on BA containing medium than NAA containing medium at both light and dark conditions. Shoot basal callus was induced during shoot multiplication procedure. Two types of callus, green colored callus and whitish - yellow colored callus, were cultured on mMS medium containing 2.0 mg/l BA and 0.5 mg/l NAA. Green colored callus showed the shoot regeneration ability while whitish - yellow callus failed to regenerate shoot and died. Regenerated shoots were rooted on hormone - free ½MS medium within 2 weeks.

Key words : Robinia pseudoacacia(*black locust*), *callus*, *shoot regeneration*, *multiple*.

요 약

기내배양된 아까시나무(*Robinia pseudoacacia* L.)의 줄기기부에서 발생된 callus로부터 기관발생을 통하여 식물체재분화 시스템을 확립하였다. Callus는 줄기를 BA 또는 NAA가 함유된 MS배지에 배양하였을 때 유도되었으며, BA처리구가 NAA처리구보다 유도율이 높았다. BA가 첨가된 배지에 줄기를 배양하였을 때 기부 callus의 생장은 광조건 및 암조건에서도 잘 증식되었다. 줄기기부에서 발생된 callus는 녹색과 희면서 노란색을 띠는 callus로 분리되어 2.0 mg/l BA와 0.5 mg/l NAA가 함유된 mMS배지에 배양하였을 때 녹색의 callus로부터 줄기가 재분화되었다. 재분화된 줄기는 생장조절제가 함유되지 않은 ½MS배지에서 발근되었다.

INTRODUCTION

Black locust(*Robinia pseudoacacia* L.) is native to the Appalachian Mountains and central hardwood forest region of the United States. It is a pioneer species which quickly develop in stands influenced by clear cutting or natural disturbances such as fire or windstorm to shade and will not develop new plants under its own canopy. Other significant characteristics of this species include indeterminate growth, tolerance of a wide range of soil qualities, and its ability to develop an extensive root system(Bridgen, 1991).

接受 1994年 11月 24日 Received on November 24, 1994.
[2] 임업협동조합중앙회 임업기술훈련원 Training & Extension Service Center for Private Forest Management N.F.C.F., Habuk - Myon, Yangsan - Gun Geongsangnam - Do, 626-860, Korea.
[3] 경북대학교 임학과 Department of Forestry, Kyungpook National University, Taegu, 702-701, Korea.

Black locust is an excellent source for the production of good dimension lumber, fencepost, honey production, etc. Black locust fixes atmospheric nitrogen and thrives on poor sites; thus it is widely planted for land reclamation purpose. It provides quick stabilization of disturbed sites and enhances soil quality by furnishing nitrogen and nutrient litter, fosters successional development of high quality forest stands(Han, 1991). This species is widely planted all over the world because of many useful characteristics. For example, in Hungary black locust occupying 18% of the country's total forest area and providing 25% of the annual timber output of the country(Keresztesi, 1988).

Black locust is also important tree in Korea since it has been imported at the end of nineteenth century. It is mostly planted for erosion control, timber, feulwood, honey production and soil improvement cooperating with *Rhizobium* which have nitrogen fixation capacity. Estimated afforestation area of black locust in Korea is 325,000 ha, but most of plantations are not managed properly. Furthermore, just few studies have been carried out for the genetic improvement of the species mostly due to its limited use in Korea.

Typically, black locust breeding programs have been targeted for fast growth, stem straightness, nectar yield and biomass production(Keresztesi, 1988).

Using conventional breeding methods biomass yields can reasonably be improved by 50 percentage with one cycle of selection. Biotechnology is the useful method to enhance the gain of genetic on breeding stratigy. Biotechnological approaches to improvement are promising to short of time(Bongarten *et al.*,1991). *In vitro* regeneration system from callus cultures are often an essential step in creating somaclonal variation for selection of desirable traits and can be used for transferring useful genes(Chalupa, 1987).

Tissue culture systems for black locust such as shoot multiplication(Chalupa, 1983; Barghchi, 1987), callus culture(Han, 1991), somatic embryogenesis(Merkle and Wiecko, 1989) and protoplast isolation and culture(Han and Keathley, 1988) were already established.

In this paper, some useful methods affecting plant regeneration from shoot basal callus(Woo, 1988) of black locust are described.

MATERIALS AND METHODS

Seeds of black locust(*Robinia pseudoacacia* L.) were obtained from Kyungpook provincial Forest Research Station in Kyung-ju, Korea. Seeds were surface sterilized step by step with 70%(v/v) ethanol for 1 min, 1%(v/v) sodium hypochlorite for 1hr, and 1%(v/v) hydrogen peroxide for 1hr and followed by rinsing with sterile distilled water. After seeds surface were scrubed with scalpel to stimulate germination they were placed on hormone-free half strength MS medium(Murashige and Skoog,1962) for aseptic germination.

The media for the experiments were MS, mMS(MS medium supplemented with 1g/l myo-inositol, 450 mg/l glutamine, 500 mg/l casein hydrolysate(CH)), GD(Gresshoff and Doy, 1972) and WPM(Lioyd and McCown, 1980) medium containing 3% sucrose as carbon source and 0.75% agar. All media were adjusted to pH 5.8 prior to autoclaving for 15 min at 121°C.

Three-week old seedlings and shoot basal callus were used as explants. Cultures were maintained at constant temperature of 25±2°C with 2,000-3,000 lux of illumination with cool white fluorescent lamps for 16 hr photoperiod or 24 hr darkness.

To investigate callus induction from various organ piece, asceptic germinated seedlings were divided into shoots, cotyledon+hypocotyl upper parts, hypocotyl middle parts, hypocotyl lower parts and roots and vertically inoculated on MS medium containing BA 1.0 mg/l or NAA 1.0 mg/l. Callus formed on various organ was dried and weighted after 1 month culture. Effects of light and darkness on callus formation were studied by inoculating shoots on MS medium containing BA 1.0 mg/l or NAA 1.0 mg/l. The culture were performed on 16 hr. of photoperiod or dark condition. Callus formed on shoot base

was dried and weighted after 1 month culture.

The procedure for shoot formation was studied by using shoot basal callus. 0.5 g of calli that have regeneration capacity were cultured on various culture media with different combination of growth regulators for shoot formation for 4 weeks. Number of shoots induced from the callus was recorded to compare the shoot forming ability among callus originated from different organs.

RESULTS AND DISCUSSION

The experiment of the effect of callus induc tion from various organ piece was resulted as followed: Shoot cultured on BA contained MS medium produced larger callus(on base) than other organ piece on BA or NAA contained medium(Fig. 1). It was peculiar phenomenon that BA stimulate the formation of shoot basal callus because BA was mostly used for shoot formation from callus or shoot multiplication (Chalupa, 1983; Barghchi and Alderson, 1985; Barghchi, 1987; Park and Son, 1988). On the experiment for the effect of light and darkness to shoot basal callus formation, shoot basal callus was larger on BA treated MS medium than on NAA treated MS medium on whether conditions of light or dark(Fig. 2). When shoot was cultured on BA treated MS medium callus may be initiated simultaneously with shoot multiplication and elongation. Similar observation was reported by Barghchi(1987) that endogenous auxin or auxin derivates may stimulate shoot basal callus formation on the shoot multiplication from cutting. But it was some different with our experiment that shoot basal callus induction was more stimulated in BA treated medium than NAA. It may be the result of BA shock which stimulate the occurrence of auxin derivates that affect the induction of shoot basal callus.

The procedure of shoot formation from shoot basal callus was as shown in Fig. 3. Firstly shoot basal callus was cultured on mMS containing BA 1.0 mg/l and Kinetin 1.0 mg/l and 2,4-D 2.0 mg/l to proliferate the callus quickly. After 1 month of culture the callus was

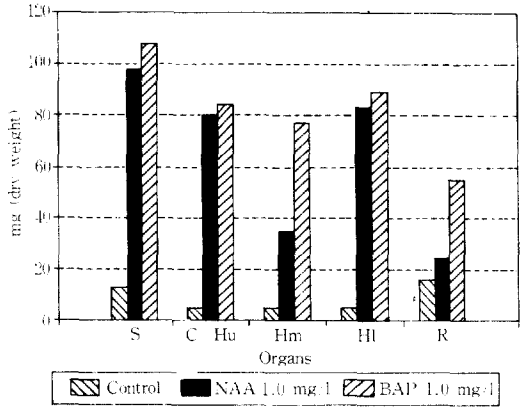

Fig. 1. Dry weight of callus produced from each organ pieces cultured on MS medium with BAP 1.0mg/l or NAA 1.0mg/l and control medium measured 4 weeks after culture. S : Shoot ; C Hu : Cotyledon + hypocotyl upper part ; Hm : Hypocotyl middle part ; Hl : Hypocotyl lower part ; R : Root

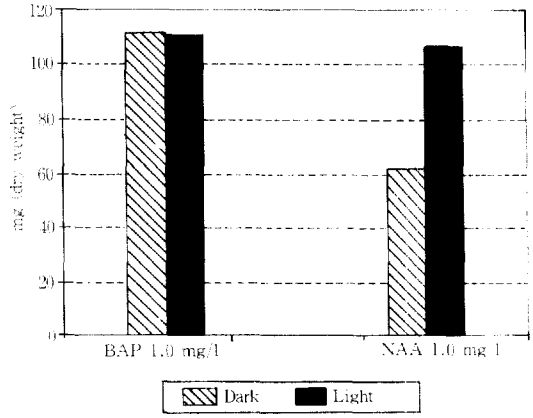

Fig. 2. Dry weight of shoot basal callus cultu red on MS basal medium affected by growth regulators and culture condi tion.

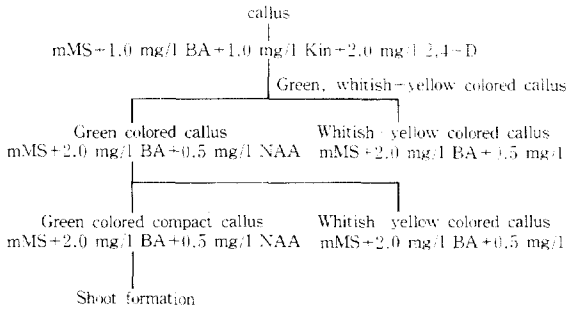

Fig. 3. Schematic diagram for organogenesis of *R. pseudoacacia* L.

separated with green colored callus and whitish - yellow colored callus and subcultured each callus to mMS supplemented with BA 2.0 mg/l and NAA 0.5 mg/l. After 1 month of culture proliferated callus were subcultured again to the same medium with separating the green colored compact callus and the whitish - yellow colored callus(Fig. 4). In the culture of green colored compact callus, it was proliferated with the change of the color of callus(green colored compact callus to brownish - green colored com - pact callus with white - yellow colored callus). Shoot formation was occurred in brownish - green colored compact callus(Fig. 4). Whitish - yellow colored callus produced no shoot and died. When the callus that may have regeneration capacity was subcultured to various medium 5 of maximum shoot number was obtained(Table 1). In our experiments callus was proliferated on high BA and NAA, shoot formation was occurred simultaneously with callus proliferation. It was similar with that shoot multiplication and elongation occurred with shoot basal callus proliferation(Woo, 1988).

Callus from cotyledon+hypocotyl upper part, hypocotyl middle part, hypocotyl lower part and

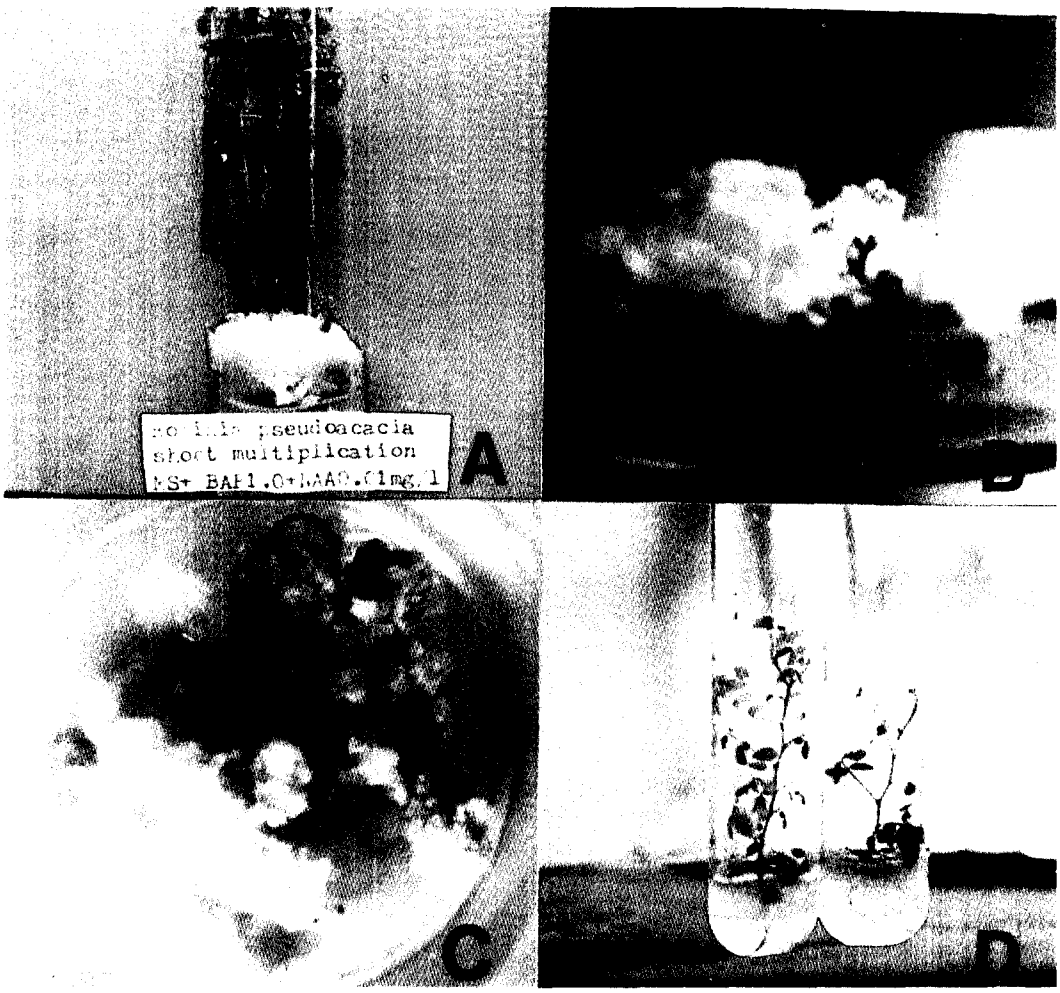

Fig. 4. Shoot regeneration from callus of black locust. A : Callus formation at multiplicated shoot base, B : shoot regeneraion from callus after 1 month of culture, C : shoot elongation of regenerated shoot, D : rooting of shoot after 1 month of culture.

Table 1. Shoot formation from shoot basal callus culture of *R. pseudoacacia* L. in various medium.

Medium	No of shoot formed callus	Shoot no(total)
MS + BA 2.0mg/l + NAA 0.50mg/l	2/9	5
mMS + BA 2.0mg/l + NAA 0.50mg/l	1/9	4
WPM + BA 0.8mg/l + NAA 0.01mg/l	2/7	4
WPM + BA 1.0mg/l + NAA 0.10mg/l	2/8	2
GD + BA 1.0mg/l + NAA 0.01mg/l	1/10	3

root were inoculated by the same procedure of Fig. 3, but the color of calluses were became white or yellow and had no green colored callus. Therefore the attempt of shoot regeneration from callus of other organ piece was failed. Pal *et al.*(1985) were reported that hard, compact and brownish-green callus of *Leucosceptrum canum* Sm. proliferated and produce shoot. In our experiment compact and green colored callus only was proliferated to brownish green colored compact callus and produced shoot.

Regenerated shoots were rooted on none hormon treated $\frac{1}{2}$ MS medium for 2 weeks(Fig. 4). In some cases shoots were produced basal callus in rooting stage. Further experiment is needed to find the unique physiological character and introduce useful genes to black locust.

REFERENCES

1. Barghchi, M. 1987. Mass clonal propagation *in vitro* of *Robinia pseudoacacia* L.(black locust) cv. 'Jaszkiseri'. Plant Sci. 53:183-189.
2. Barghchi, M. and P.G. Alderson. 1985. *In vitro* propagation of *Pistacia vera* L. and the commercial cultivars Ohade and Kalleghoch. J. Horticultural Sci. 60: 423-430.
3. Bongarten, B.C., S.A. Merkle and J.W. Hanover. 1991. Genetically improved black locust for biomass production in short rotation plantations. *in*: Energy from Biomass and Wastes x v, edited by D.L. Klass, Inst. Gas Technology, Chicago, U.S.A. pp. 391-409.
4. Bridgen, M.R. 1992. Plantation silviculture of black locust. *in*: Proceedings: International Conference on Black Locust: Biology, Culture, & Utilization, eds. Hanover, J.W., K. Miller and S. Plesko, June. 17-21, 1991, Kellogg Center for Continuing Education, Michigan State Univ., East Lansing, Michigan, U.S.A. pp. 21-32.
5. Chalupa, V. 1983. *In vitro* propagation of willow(*Salix* spp.), european mountain-ash(*Sorbus aucuparis* L.) and black locust(*Robinia pseudoacacia* L). Biol. Plant. 25: 305-307.
6. Chalupa, V. 1987. European hardwoods. *in*: Tissue culture in forestry, vol 3, Bonga, J. M. and Durzan, D.J. eds. Martinus Nijhoff publishers, pp.224-246.
7. Gresshoff, P.M. and C.H. Doy. 1972. Development and differentiation of haploid *Lycopersicon esculenium* (tomato). Planta 107:161-170
8. Han, K.H. 1991. *Agrobacterium rhizogenes* mediated transformation and plant regeneration from normal and transgenic tissues of *Robinia pseudoacacia* L. Thesis of Ph. D., Michigan State Univ., U.S.A.
9. Han, K.H. and D.E. Keathley. 1988. Isolation and culture of protoplasts from callus tissue of black locust(*Robinia pseudoacacia* L.). Nitrogen Fixing Tree Res. Rep. 6:68-70.
10. Keresztesi, B. 1988. The black locust. Akademiai Kiado Budapest, Hungary.
11. Lloyd, G. and B. McCown. 1981. Commercially feasible micropropagation of mountain laurel(*Kalmia latifolia*) by use of shoot-tip culture. Proc. Intl'. Plant Prop. Soc. 30:421-427.
12. Merkle, S.A. and A.T. Wiecko. 1989. Regeneration of *Robinia pseudoacacia* via somatic embryogenesis. Can. J. For. Res.

19: 285-288.

13. Mhatre, M., V.A. Bapat and P.S. Rao. 1985. Regeneration of plant from the culture of leaves and axillary buds in mullbery (*Morus indica* L.). Plant Cell Reports 4: 78-80.
14. Murashige, T. and F. Skoog. 1962. A revised medium of rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
15. Pal, A., A. Banerjee and K. Dhar. 1985. *In vitro* organogenesis and somatic embryogenesis from leaf explants of *Leucosceptrum canum* Sm. Plant Cell Reports 4: 281-284.
16. Park, Y.G. and S.H. Son. 1988. *In vitro* organogenesis and somatic embryogenesis from punctured leaf of *Populus nigra* · *P. maximmowiczii*. Plant Cell, Tissue and Organ Culture 15: 95-105.
17. Woo, J.H. 1988. Tissue culture of *Robinia pseudoacacia* L. - Mass propagation, callus and cell suspension culture, protoplast isolation and culture. Thesis of Master, Kyungpook Natl'. Univ., Korea.