

Effect of Some Factors Affecting Callus Formation of Red Clover

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레드 클로버의 캘러스 誘導에 미치는 몇가지 要因의 影響

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摘 要

다섯가지 품종의 레드 클로버의 캘러스 유도에 미치는 몇가지 요인을 밝히기 위하여 본 실험을 행하였으며, 얻어진 결과는 아래와 같았다.

Callus 유도에서 auxin원으로서 0.1 ml/l의 picloram이 가장 효과적인 것으로 나타났으며, 여러 종류의 배지중에서는 PC(Phillips & Collins) 배지가 가장 callus 형성이 좋았다. PC 배지에서 생성된 callus는 다른 배지에서 생선된 callus보다 더 무른 것이 특징이었다. 배지의 pH는 5.8이 가장 좋았으며 6.2 이상에서는 callus의 형성이 급격히 나빠졌다. Explant 재료로서의 식물체는 5~7일령이 가장 좋았고, 15일령 이상은 매우 좋지 못했다. Agar농도도 callus의 형성에 영향을 미쳤는데, 0.5~0.7%가 가장 좋았고 1% 이상에서는 callus형성이 매우 좋지 못하였다.

I. INTRODUCTION

Leguminous plants are generally intolerable to soil acidity(Jo et al., 1980). Red clover (*Trifolium pratense* L) grows moderately on acid soils but maximum yields are obtained only when Ca is adequate and soil pH is 6 or higher(Taylor, 1978). Acidic soil prevails in the grassland areas of Korea(average pH 5.3 ± 0.6)(Hwang, 1973). For amelioration of these hazardous effects by soil acidity, acidic soils can be neutralized by liming. Liming the whole layer of grassland soil where radicles are distributed is laborious work. Accordingly, the acid tolerant variety is necessary to cultivate red clover in acidic soils. This experiment was conducted to select acid tolerant cell lines which will lead to the acid tolerant red clover plant.

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II. MATERIALS AND METHODS

1. Plant Material

In all of this experiment, Redman red clover was used except for variety compariosn. Five varieties of Redland, Redman, Atlas, Kenland and Flare were compared. Seeds were sterilized in 70% ethanol for 3 minutes, 1% sodium hypochlorite for 10 minutes, 1% hydrogen peroxide for 3 minutes and were washed in sterilized and distilled water. They were germinated in the dark at 28°C on the PC basal medium(Phillips and Collins) solidified with 0.7% agar containing 25% sucrose for 5 days. They were illuminated under 1,500 lux of flourescent light with a 16 photoperiod for 3 days. Eight-day old seedlings were dissected into cotyledons and hypocotyls. Intact cotyledons and 10mm cut

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hypocotyls were used as explants.

2. Callus formation

Growth regulators were compared among the combinations of picloram(4-amino-3, 5, 6-trichloropicolinic acid) and 2, 4-D(2, 4-dichlorophenoxy acetic acid) as auxin source and BAP(6-benzyl aminopurine) and 2ip {6-(*r,r*-dimethylallylamino) purine} as cytokinin source. Basal media were compared among BO(Blaydes), B₅ (Gamborg), MS(Murashige & Skoog), PC(Phillips & Collins) and SH(Schenk & Hildebrandt). Medium pH, the age of plant used as explant material, and agar concentration were also investigated. All of the treatments were replicated six times and the data were arranged statistically in this experiment.

III. RESULTS AND DISCUSSION

1. Effect of growth regulators

The effect of concentrations and combinations of growth regulators for callus formation in Redman red clover is shown in Figure 1. The highest callus yield was achieved with 120 mg/petri dish in the single treatment of 0.1 mg/ℓ picloram from hypocotyl explant. The second highest yield was 101 mg/petri dish in 2 mg/ℓ 2, 4-D+0.1 2ip mg/ℓ treatment and the third was 97 mg/petri dish in the 0.1 mg/ℓ picloram+ 0.1mg/ℓ BAP. The significant difference was observed not only among the all treatments but among the treatments of the three highest yields($P<0.05$). Single

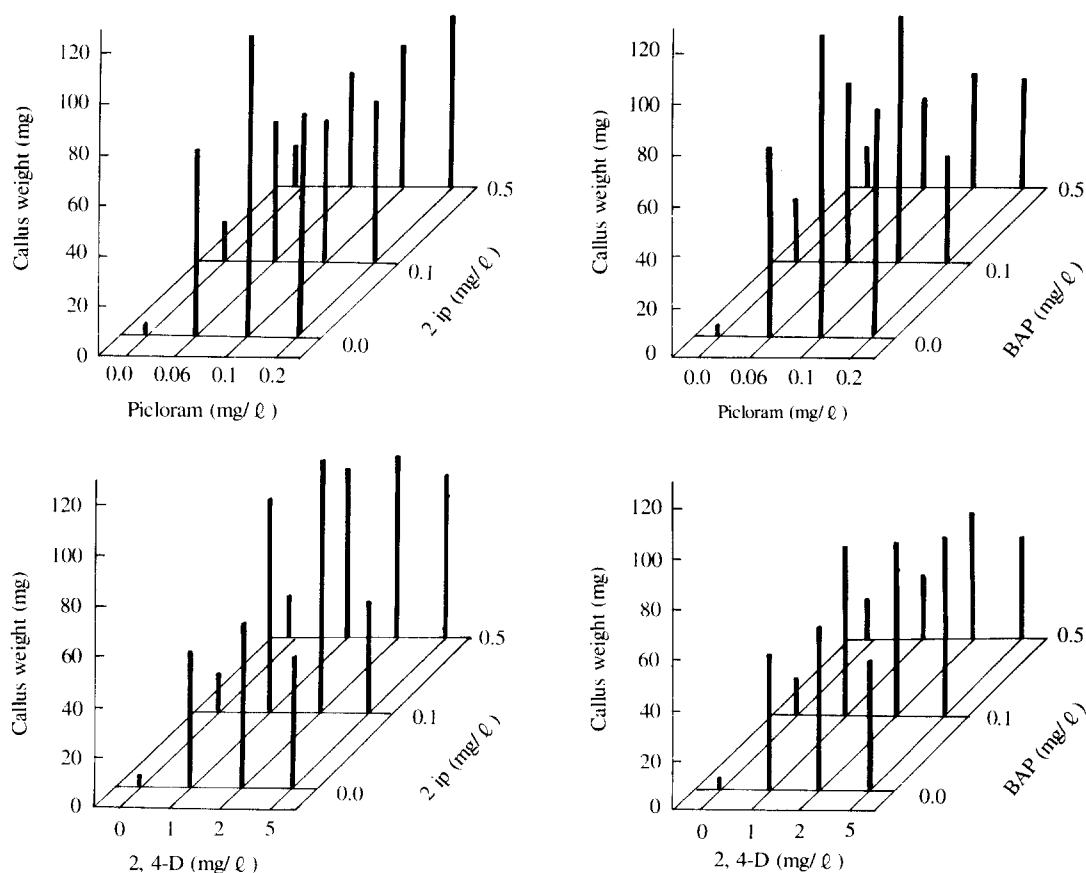


Fig. 1. Effect of concentrations and combinations of growth regulators for callus formation in Redman red clover

treatment of auxin source such as 2, 4-D or picloram derived fairly good callus formation, whereas single treatment of cytokinin such as BAP or 2ip barely induced callus. In the auxin and cytokinin combination, 2, 4-D treated with one of cytokinin source resulted better callus formation than picloram did. Cytokinins were not necessarily required for callus formation as in the previous paper for alfalfa(Yun et al., 1989), though many scientists would use low concentrations of cytokinin(Bingham & Saunders, 1975; Mitten et al., 1984; Brown & Atanassov, 1985). Phillip et al. used 0.06 mg/m³ picloram + 0.2 mg/ℓ BAP for callus formation. The difference between the result of this experiment and that of Phillip et al. was thought to be

brought from the difference of the varieties used. The callus formation was different not only from variety to variety but also from genotype to genotype(data not shown).

2. Effect of growth medium

Callus formation according to growth medium is shown in Table 1. The highest yield was obtained in PC medium with 117 mg fresh weight per petri dish, though the significant difference was not observed among medium treatments. Phillip et al. reported the best result in callus formation had appeared in PC medium but no difference among media had been observed after several subcultures(Phillip et al., 1980).

Table 1. Effect of various media on the callus formation of Redman red clover

Media	PC-L2	SH	MS	Blaydes	B5	LSD(0.05)
Callus weight(mg)	117 ^{a*}	109 ^a	105 ^a	107 ^a	112 ^a	13.1

* Values followed by different letters are significantly different at P = 0.05.

3. Effect of other factors

Optimum pH for callus formation was investigated as shown in Table 2. The highest callus yield was obtained in pH 5.8 treatment with 120 mg/petri dish and it was well corresponded with previous paper for Vernal alfalfa(Yun et al., 1989). The second highest yield was 101 mg/petri dish in pH 5.4 treatment and the yield was significantly different from pH 5.8 treatment(P<0.05). Murashige and Skoog(1962) reported that tissue cultured

cells had grown favorably in the pH range of 5.2~5.8 before autoclaving in which pH minerals added in the medium had dissolved most vigorously. In the pH above 7.0, callus was hardly induced and the tissues bedded turned brown rapidly. They recommended to heat the medium for few minutes before pH adjustment because this prevented considerable pH shift during autoclaving. They found the pH range of 5.7~5.8 was suitable to maintain all the salts in the soluble form even with high phosphate level.

Table 2. Effect of medium pH on the callus formation of Redman red clover

pH	5.0	5.4	5.8	6.2	6.6	LSD(0.05)
Callus weight(mg)	120 ^{a*}	87 ^{bc}	101 ^b	85 ^c	67 ^d	13.9

* Values followed by different letters are significantly different at P = 0.05.

In the Table 3, agar concentrations were compared for callus formation. The highest yield was 110 mg/petri dish in the 0.5% agar concentration but among three treatments of 0.0, 0.5 and 0.7%, there was no difference. In 1% treatment, callus formation decreased significantly

(P<0.05). The favorable callus formation in the lower than 0.7% agar concentration was thought to be increased availability of ingredients on agar because of lower water potential. In the agar concentration lower than 0.8%, callus induced was white and friable while

that induced in the higher agar concentration was hard, dry and redish brown. George and Sherrington(1984) deduced that large molecules was hard to diffuse in the higher agar concentration and that the decreased callus growth of callus in the higher agar concentration and

that the decreased callus growth of callus in the higher agar concentration was because of immobilization of invertases released from cultured cells. Invertase immobilization would result in a reduced availability of glucose in the cultured cells.

Table 3. Effect of plant age on the callus formation of Redman red clover

Plant age(days)	5	7	9	15	20	LSD(0.05)
Callus weight(mg)	120*	109 ^a	107 ^a	82 ^b	57 ^c	12.2

* Values followed by different letters are significantly different at P = 0.05.

Callus formation was also influenced by the age of plant that the explant were adopted. Younger plants were better material for explant than older ones. In an experiment with Redman red clover, explants from 5-day old plants resulted the best callus formation with 120 mg fresh weight/petri dish but among three treatments within 9 days of age there were no difference. Callus formation was significantly decreased in hypocotyl explants with ages over 15 days. George and Sherrington(1984) discussed better callus formation

in young plant material for explant was because of vigorous cell proliferation. Among red clover varieties of Redland, Redman, Atlas, Kenland and Flare, no significant difference was observed as shown in Table 4. Though the variety difference was not observed in this experiment, the varieties must be discriminated in the subsequent experiments according to the experiment purpose. Generally the ability of embryogenesis is quite different among the varieties in other species.

Table 4. Comparison of callus formation ability among the red clover varieties

Variety	Redman	Atlas	Kenland	Flare	Redland II	LSD(0.05)
Callus weight(mg)	113**	118 ^a	123 ^a	115 ^b	110 ^a	12.7

* Values followed by different letters are significantly different at P = 0.05.

IV. SUMMARY

This experiment was conducted to determine factors affecting callus formation of some varieties of red clover. Growth regulators, basal medium, medium pH, agar concentration, and plant age for explant were investigated. The result obtained were as follows.

As auxin source, 0.1 mg/ℓ picloram was found to be effective on callus formation. Callus formation was the highest on PC medium among several growth media. The callus produced on PC medium was more friable than those produced on the other media.

The medium pH 5.8 gave the best response for callus formation. The effective seedling age for callus

formation was around 5~7 days old. In agar concentration, 0.5~0.8%(W/V) was suitable for callus formation.

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