Effect of Amino Acids on Callus Induction from Bentgrass

Ming Liang Chai, Doo Hwan Kim*

Department of Horticultural Science, Konkuk University

아미노산이 Bentgrass 캘러스 유도에 미치는 영향

Ming Liang Chai · 김두환*

건국대학교 원예과학과

ABSTRACT

The effect of proline, glutamine, aspartic acid and their combinations on callus induction and embryogenic callus formation from 3 creeping bentgrass (*Agrostis palustris*) cv. Regent, Mariner, Cato and 1 colonial bentgrass (*Agrostis tenuis*) cv. Tiger was estimated in both light and dark condition. The addition of amino acids to the growth medium did not have a significant stimulatory effect on the induction of embryogenic callus, instead, they were inhibitory, particularly at higher concentration (40 mM). But supplement of amino acids at lower concentrations (5 or 10 mM) to basal medium was beneficial in inhibiting the formation of hairy outgrowth on the surface of embryogenic callus.

Key words: amino acid, bentgrass, colonial, creeping, callus

INTRODUCTION

Bentgrass (*Agrostis spp.*), especially creeping bentgrass (*Agrostis palustris*) is one of the most important cool season grasses and is used primarily on golf course putting greens and tees, bowling greens, and grass tennis courts. Creeping bentgrass produces a fine-textured, soft, extremely dense, carpet-like sod. Its quality surpasses that of any other northern turfgrass. But creeping bentgrass is susceptible to many kinds of diseases, such as dollar spot (*Sclerotina homeocarpa*), brown patch (*Rhizoctonia salani*), pythium blight (*Pythium graminicola*) and also more prone to herbicide injury. Traditional breeding method has the limitation for improving resistance to these diseases, so recently much effort has been diverted to develop improved varieties using biotechnological approaches.

Induction of high quality embryogenic callus is the prerequisite in developing geneti-

*corresponding author

cally modified monocots. Although callus induction and regeneration of creeping bentgrass has been established (Krans, et al., 1982; Blanche et al., 1986; Zhong, et al., 1991; Terakawa, et al., 1992), systematic research on specific cultivars is still necessary for any laboratory before initiating transformation experiment.

One method that has been used for embryogenic callus induction is to supplement the medium with organic nitrogen sources, especially amino acids (Armstrong et al., 1985; Nutri-Ronchi et al., 1984; Trigiano et al., 1987; Shetty et al., 1991). But the results were unstable and varying depending on the plant species, kinds of amino acids and their concentrations. So, in this study the function of several amino acids on callus induction from bentgrass was examined in order to enhance efficiency of embryoenic callus induction.

MATERIALS AND METHODS

Commercial seeds of three creeping bentgrass (*Agrostis palustris* Huds) cv. Regent, Mariner and Cato (Pickseed West Inc., USA) and one colonial bentgrass (*Agrostis tenuis*) cv. Tiger (International Seeds Inc., USA) were used as plant materials. The seeds were surface sterilized with 70 % (v/v) ethanol for 1 min followed by treatment with 0.1 % (w/v) $HgCl_2$ for 20 min. The seeds were rinsed with sterile water 3 times and placed on the surface of MS medium (Murashige, et al., 1962) with 2 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid) for the induction of callus. Various concentrations of proline, glutamine and aspartic acid and their combinations were also added and the pH adjusted to 5.8 with 0.5 N NaOH or 0.5 N HCl. Phytagel at 0.3 % (w/v) was used as solidifying agent. The medium was sterilized by autoclaving under standard condition for 15 min at 121 ℃.

Embryogenic callus was determined after 6 weeks of incubation in dark or light (16 h light period, 1500 lux) at 25±2 ℃. Embryogenic callus was yellowish ('Regent', 'Mariner' and 'Cato') or white opaque ('Tiger'), nodular, compact or friable. Every treatment had 2 or 3 petri-dishes (90 mm in diameter), each contained 80 seeds. Callus percentage is represented as the ration of total calli induced to the total number of seeds inoculated and embryogenic callus percentage as the ratio of number of embryogenic calli to total seeds inoculated. The size of callus is marked (+) for small, (++) for medium and (+++) for large sized calli.

RESULTS AND DISCUSSION

All the three amino acids tested had no significant stimulatory effect on the rate of callus induction and embryogenic callus formation from 'Regent' (Table 1, 2 and 3). It

Table 1. Effect of proline on callus induction from 'Regent'

Proline concentration (mM)	Light condition	No. of inoculated seeds	callus (%)*	embryogenic callus (%)*	Callus size
0	light	240	188(78.33)	84(35.00)	++
	dark	240	190(79.17)	87(36.25)	+++
5	light	240	187(77.91)	83(34.58)	++
	dark	240	192(80.00)	90(37.50)	+++
10	light	240	185(77.08)	85(35.42)	+
	dark	240	190(79.17)	89(37.08)	++
20	light	240	178(74.16)	87(36.25)	+
	dark	240	180(75.00)	88(33.67)	++
40	light	240	170(70.83)	80(33.33)	+
	dark	240	156(65.00)	58(24.17)	+

Table 2. Effect of glutamine on callus induction from 'Regent'

Glutamine concentration (mM)	Light condition	No. of inoculated seeds	No. of Callus (%)	No. of Embryogenic callus (%)	Callus size
0	light	240	196(81.66)	90(37.50)	++
	dark	240	199(82.91)	94(39.16)	+++
5	light	240	187(77.91)	85(35.41)	++
	dark	240	178(74.16)	85(35.41)	+++
10	light	240	181(75.41)	83(34.58)	+
	dark	240	186(77.50)	83(34.58)	++
20	light	240	186(77.50)	95(39.58)	+
	dark	240	188(78.33)	89(37.08)	++
40	light	240	168(70.00)	90(37.50)	+
	dark	240	140(60.41)	46(19.16)	+

Table 3. Effect of aspartic acid on callus induction from 'Regent'

Aspartic acid concentration (mM)	Light condition	No. of inoculated seeds	No. of callus (%)	No. of embryogenic callus	Callus size
0	light	240	208(86.66)	77(32.08)	++
	dark	240	203(84.58)	95(39.58)	+++
5	light	240	208(86.66)	88(36.66)	++
	dark	240	207(86.25)	89(37.08)	+++
10	light	240	206(85.83)	103(42.19)	+
	dark	240	203(84.58)	93(38.75)	++
20	light	240	202(84.16)	85(35.41)	+
	dark	240	201(83.75)	71(29.58)	++
40	light	240	194(80.83)	87(36.25)	+
	dark	80	63(78.75)	25(31.25)	+

was observed that at lower concentrations (5 or 10 mM) hairy outgrowth on the surface of callus was inhibited and so was beneficial for subculture. At higher concentrations (40 mM) callus induction and embryogenic callus formation were inhibited and this phenomenon was very clear in the presence of glutamine and proline under dark. To some extend our results were similar to the work of Shetty, et al., (1991) in *Agrostis alba* L. The supplement of proline in culture medium stimulated somatic embryogenesis in maize (Armstrong et al., 1985) and carrot (Nuti-Ronchi et al., 1984) which is contradictory to our results. In *Gossypium klotzschianum*, glutamine promoted somatic embryogenesis in suspension cultures (Price and Smith, 1979).

Combination of amino acids also showed the similar results (Table 4, 5, 6, and 7). Combination of 3 amino acids inhibited embryogenic callus formation especially in dark. In orchard grass a combination of proline and serine stimulated somatic embryogenesis (Trigiano and Conger, 1987). The variability in results from plant to plant reflects the genotypic-dependent reaction to amino acid. The exact stimulating mechanism is not

Table 4. Effect of amino acid combination on callus induction from 'Regent'

Amino acid and concentration (mM)	Light condition	No. of inoculated seeds	No. of callus (%)	No. of embryogenic callus (%)	Callus size
0	light	160	143(89.37)	59(36.87)	+
	dark	160	144(90.00)	59(36.87)	++
Aspartic acid 2 mM	light	160	154(96.25)	59(36.87)	+
	dark	160	149(93.12)	64(40.00)	++
Aspartic acid 2 mM + proline 10 mM	light	160	139(86.87)	58(36.25)	+
	dark	160	150(93.75)	64(40.00)	++
Aspartic acid 2 mM + proline 10 mM + glutamine 6 mM	light	160	142(88.75)	53(33.12)	+
	dark	160	140(87.50)	43(26.87)	+

Table 5. Effect of amino acid combination on callus induction from 'Mariner'

Amino acid and *concentration*	Light *condition*	No. of inoculated seeds	No. of callus (%)	No. of embryogenic callus (%)	Callus size
0	light	160	107(66.87)	41(25.62)	+
	dark	160	116(72.50)	58(36.25)	++
Aspartic acid 2 mM	light	160	115(71.87)	54(33.75)	+
	dark	160	125(78.12)	59(36.87)	++
Aspartic acid 2 mM + proline 10 mM	light	160	108(67.50)	47(29.37)	+
	dark	160	115(71.87)	54(33.75)	++
Aspartic acid 2 mM + proline 10 mM + glutamine 6 mM	light	160	102(63.75)	51(31.87)	+
	dark	160	116(72.50)	42(26.25)	+

Table 6. Effect of amino acid combination on callus induction from 'Cato'

Amino acid and concentration (mM)	Light condition	No. of inoculated seeds	No. of callus (%)	No. of embryogenic callus (%)	Callus size
0	light	160	119(74.37)	54(33.75)	+
	dark	160	122(76.25)	54(33.75)	++
Aspartic acid	light	160	119(74.37)	43(26.87)	+
	dark	160	122(76.25)	53(33.12)	++
Aspartic acid 2mM + proline 10 mM	light	160	97(60.62)	48(30.00)	+
	dark	160	118(73.75)	44(27.50)	++
Aspartic acid 2 mM + proline 10 mM + glutamine 6 mM	light	160	96(60.00)	42(26.25)	+
	dark	160	108(67.50)	35(21.87)	+

Table 7. Effect of amino acid combination on callus induction from 'Tiger'

Amino acid and concentration	Light condition	No. of inoculated seeds	No. of callus (%)	No. of embryogenic callus(%)	Callus size
0	light	80	78(97.50)	31(38.75)	+
	dark	160	156(97.50)	61(38.12)	++
Aspartic acid 2 mM	light	160	154(96.25)	65(40.62)	+
	dark	160	158(98.75)	55(34.37)	++
Aspartic acid 2 mM+ Proline 10 mM	light	160	153(95.62)	62(38.75)	+
	dark	160	159(99.37)	60(37.5)	++
Aspartic acid 2 mM+ Proline 10 mM+ glutamine 6 mM	light	160	155(98.12)	55(34.37)	+
	dark	160	155(98.12)	51(31.87)	+

known. For the function of proline, Nuti-Ronchi et al. (1984) have hypothesized that it could be through : 1) regulation of osmotic balance, 2) regulation of mitosis, and 3) regulation via hydrovy-proline-rich glycoproteins involved in auxin related growth regulation. Hence, further intense research is necessary for examining the mechanism of action of amino acids to callus induction and embryogenic callus formation.

REFERENCES

Armstrong, C. L. and C. E. Green. 1985. Establishment and maintenance of friable, embryogenic maize callus and the involvement of L-proline. Planta 164:207-214.

Blanche, F. C., J. V. Krans, and G. E. Coats. 1986. Improvement in callus growth and plantlet formation in creeping bentgrass. Crop Sci. 26:1245-1248.

Krans, J. V., V. T. Henning and K. C. Torres. 1982. Callus induction, maintenance and plantlet regeneration in creeping bentgrass. Crop Sci. 1193-1197.

Koetje, D. S., H. D. Grimes, Y. C. Wang, and T. K. Hodges. 1989. Regeneration of indica rice (*Oryza sativa* L.) from primary callus derived from immature embryos. J. Plant Physiol. 135:184-190.

Nuti-Ronchi, V., M. A. Caligo, M. Nozzolini, and G. Luccarini. 1984. Stimulation of carrot somatic embryogenesis by proline and serine. Plant Cell Rep. 3:210-214.

Price, H. J. and R. H. Smith. 1979. Somatic embryogenesis in suspension cultures of *Gossypium klotzschianum* Anderess. Planta 145:305-307.

Shetty, K. abd Y. Asano. 1991. The influence of organic nitrogen sources on the induction of embryogenic callus in *Agrostis alba* L. J Plant Physiol. 139:82-85.

Terakawa, T., T. Sato, M. Koike. 1992. Plant regeneration from protoplasts isolated from embryogenic suspension cultures of creeping bentgrass (*Agrostis palustris* Hunds.). Plant Cell Rep. 11:457-461.

Trigiano, R. N., B. V. Conger. 1987. Regulation of growth and somatic embryogenesis by proline and serinein suspension culture of *Dactylis glomerata*. J. Plant Physiol. 130:49-55.

Zhong, H., C. Srinivasan, and M. B. Sticklen. 1991. plant regeneration via somatic embryogenesis in creeping bentgrass (*Agrostis palustris* Huds.). Plant Cell Rep. 10:453-456.

요 약

Creeping bentgrass인 Regent, Marine, Cato와 colonal betgrass인 Tiger 품종에서 광, 또는 암조건하에 proline, glutamine, aspartic 산과 이들간의 조합이 캘러스 유도 및 배발생 캘러스 형성에 미치는 영향을 측정하였다. 아미노산은 배발생 캘러스 유도에 크게 영향을 미치지 않은 반면에 40mM의 고농도에서는 억제효과를 가져왔다.

저농도(5~10mM)로의 첨가는 계대배양시 장해가 되는 hairy material 발생을 억제시키는 효과가 있었다. 광조건하에서 캘러스는 상대적으로 작고 쉽게 재분화되었으며 배발생 캘러스 부분은 노랗거나 초록색을 띄어 쉽게 판별이 되었다.