P87. Selection of 5-Methyltryptophan and S-(2-Aminoethyl)-L-Cysteine Resistant Cell Lines from Anther Cultures Irradiated with Gamma Rays

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Objectives

This report was performed for the purpose of selection of free amino acids overproducing cell lines resistant to 5-methyltryptophan (5MT) or S-(2-aminoethyl)-L-cysteine (AEC) from a haoploid cell culture system of *Oryza sativa* L..

Materials and Methods

◇ Plant material : *Oryza sativa* L. cv. Ilpumbyeo

◇ Haploid callus induction medium : N6 basal medium with 1 ppm NAA

◇ Selection medium : AA medium with 1 ppm 2,4-D, 0.1 ppm GA_3, and 0.2 ppm kinetin

◇ Amino acid analogs : 5-methyltryptophan (5MT) and S-(2-aminoethyl)-L-cysteine (AEC)

◇ Mutagen : 120 Gy for 5MT and 30 Gy Gamma rays for AEC resistant cell selection

◇ Free amino acid analysis : Waters Pico-Tag method using Waters HPLC system

Results

Haploid cell lines resistant to 5MT (a tryptophan analog) or AEC (a lysine analog) were selected in rice by *in vitro* mutgenesis. For selection of 5MT resistant cell lines, suspention-cultured cells were plated on AA solid medium supplemented with a concentration (250 μM) of 5MT. Thirteen 5MT resistant cell lines were selected and they were able to grow stably at 500 μM 5MT. Analysis of the free amino acids in five callus lines (MR12-1 to MR12-5) showed a 7.4 to 46.6 times greater level than in the wild type culture. Tryptophan, phenylalanine, and tyrosine levels in the shikimate pathway were 28.1 and 22.5 times higher than the control callus in MR12-3 and MR12-4, respectively.

Four AEC resistant cell lines were isolated from 1 mM AEC by screening irradiated haploid cells. They were able to grow stably at 2 mM AEC and accumulated free lysine at levels 2.2 to 12.9-fold higher than in controls. Especially, the levels of aspartate, asparagine, and methionine in aspartate pathway showed higher in the AEC resistant lines than in the control callus.

These results indicated the 5MT or AEC resistant cell lines are useful in studies of amino acid biosynthesis.

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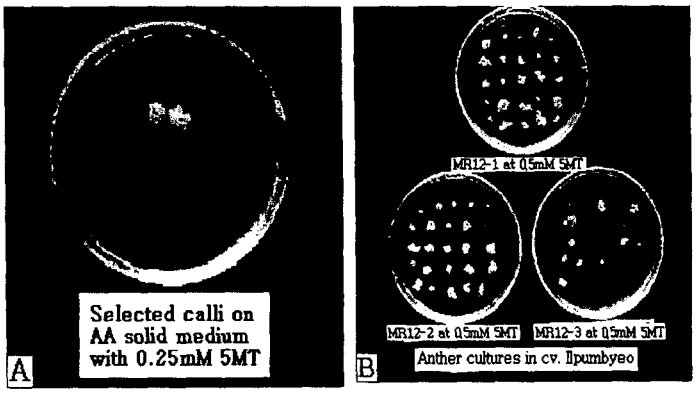

Fig. 1. Selection of 5MT-resistant haploid cells from Ilpumbyeo anther cultures. A) 5MT resistant cells selected from AA selection medium containing 0.25 mM 5MT after irradiation with 120 Gy gamma rays, B) re-screening of haploid 5MT-resistant cell lines selected from 0.25 mM 5MT at 0.5 mM 5MT.

Table 1. Free amino acid profiles of control and 5MT resistant anther cultures in *Oryza sativa* L. cv. Ilpumbyeo.

Amino Acid	Contents of 5MT resistant cell lines (n mole/ g fw.)					
	Control	MR12-1	MR12-2	MR12-3	MR12-4	MR12-5
Cys	24.31	184.70	60.00	440.69	393.55	321.52
Asp	31.10	851.35	311.08	2051.68	1028.60	987.00
Glu	88.92	1892.79	223.39	2536.84	1910.13	2315.82
Asn	37.49	456.44	975.75	55.11	1114.85	705.26
Ser	34.40	842.44	588.20	2618.80	993.91	1132.79
Gln	19.59	530.52	170.83	605.62	419.25	720.85
Gly	41.80	623.05	292.75	935.02	695.64	998.70
His*	59.06	769.15	289.67	303.39	787.86	1658.64
Arg	19.93	765.95	95.50	2159.71	1297.51	1517.59
Thr*	26.33	707.93	248.10	702.52	689.61	837.02
Ala	61.74	3442.68	308.94	8691.73	3364.64	5995.74
Pro	46.34	751.20	153.37	1031.64	575.05	1344.36
Tyr	14.39	263.07	125.07	576.06	450.48	295.90
Val*	31.25	731.94	246.54	1248.64	909.45	1012.46
Met*	7.18	314.27	60.54	837.18	529.89	407.26
Ile*	16.67	350.93	68.66	752.16	544.73	437.99
Leu*	33.43	599.90	65.91	2057.84	1228.55	504.94
Phe*	29.58	354.28	249.95	748.52	652.61	384.79
Trp*	18.49	93.15	60.78	428.19	301.30	87.17
Lys*	14.09	838.13	199.22	1491.69	1111.95	1045.68
Total	650.26	15363.78	4794.26	30273.06	18999.56	22711.51
Ratio†		23.63	7.37	46.56	29.22	34.93

* Essential amino acid

† Ratio of MR12 lines/Control callus