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サトウキビのカルス急照射による再分化系統群の推定染色体数と農業形質の関係

Association among estimated chromosome numbers and agronomic characteristics of regenerated mutant lines derived from acute gamma-irradiated calli in sugarcane

培養系を用いた放射線育種では，培養再分化個体・系統に及ぼす照射法の効果が顕著に現れることが多い。そのため，照射法による再分化系統群の特性の変化を解析して，効率的な方法を確立する必要がある。サトウキビの培養体へガンマ線を急照射して再分化した変異系統を対象に，線量と系統の農業形質との関係について解析を行った。核 DNA 量は染色体数と密接な関係があるため，系統の放射線障害による染色体の変化を解析する手段として検討した。

方法：サトウキビ品種 Ni 1 を用いて，幼葉を培養外植片としてカルスを誘導し，ガンマールームにおいて，50Gy ～ 500Gy の範囲で，20 時間の急照射を行った。その後カルスを培地に移植し，再分化個体を養成し圃場に定植した。各個体の栄養系に由来した 111 系統を供試し，収量，品質に関する形質を調査した。フローサイトメーター（PARTEC）を用いて，品種 Badila と系統の葉片を混合して核 DNA 量を測定した。また Badila（2n ＝ 80）を基準にして，各系統の推定染色体数を算出した。

結果の概要：サトウキビ品種は高次の倍数性，異数性で，染色体モザイク性を示すため，正確な染色体数の測定は困難である。上述の方法により原品種 Ni 1 の染色体数は 2n ＝ 116 と推定された。無照射区では，原品種の推定染色体数との間では差がないが，照射区では線量が増加するにつれて顕著に減少した（図 2）。線量と染色体数との間には有意な負の相関係数があり，両者の回帰分析によれば，100Gy の急照射量に対して 3.3 本相当の染色体が減少した。500Gy の照射では，16 本相当の染色体が減少し，生存の限界であった。

培養再分化系統では，一般に急照射による線量の増加に伴い生長量が減少した（図 1）。染色体数と葉や茎のサイズ，茎の収量との間には高い正の相関があり，糖分との間には負の相関があった（表 1）。つまり再分化系統は照射により推定染色体数が減少し，全体的にはバイオマスも減少した。

一方，一般的形質の変異幅について，0Gy では変異幅は狭いが，50Gy，100Gy では変異幅が広くなり，200Gy ではやや狭くなり，300Gy 以上では低位に収斂した。原品種よりも生育の良い系統は 50 ～ 200Gy の照射区に現れ，適正な照射により系統の変異幅を拡大する効果が一部認められた。

なお適正な照射法としては，放射線障害を極力避け，染色体数の減少を最小限に抑えながら，収量・糖分・形態の変異拡大を図ることが肝要であり，それにより多収性・高品質の変異体の誘発は可能と考えられる。

（永冨成紀・出花幸之介）

図 1　サトウキビの葉片由来カルスへの急照射による再分化系統の生育特性。
右から，原品種，Ni1（1 鉢），0Gy 区（3 鉢），50Gy（3），100Gy（3），200Gy（3），300Gy（3），500Gy（1）

Fig. 1. Growth habit of clonal lines of sugarcane regenerated from leaf-induced calli irradiated with acute gamma rays. From right to left: Original variety, Ni 1 (1 pot), 0Gy (3), 50Gy (3), 100Gy (3), 200Gy (3), 300Gy (3), and 500Gy(1).

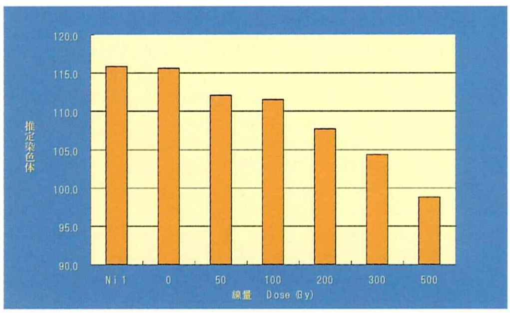

図 2　サトウキビの葉片カルスへの急照射による再分化系統の平均の推定染色体数。

Fig. 2. Average estimated chromosome number of clonal lines of sugarcane regenerated from leaf-induced calli irradiated with acute gamma rays.

表１　サトウキビのカルス急照射培養による再分化系統の主要形質の変異

Table 1. Variation of major characteristics of clonal lines of sugarcane regenerated from leaf-induced calli irradiated with gamma rays.

線量 Dose	系統数 No. of clones	蔗茎収量 Cane yield (kg/a)	比率 Ratio	ブリックス Brix (%)	糖量 Sugar yield (kg/a)
Ni 1 (Or)	1	680.0	100	14.55	98.94
0 Gy	31	585.5	86	13.22	77.42
50 Gy	26	508.9	75	13.66	69.53
100Gy	23	509.2	75	14.79	75.32
200Gy	22	482.6	71	15.60	75.30
300Gy	8	370.5	54	14.53	53.84
500Gy	1	303.0	45	14.55	44.09

表２　サトウキビのカルス急照射培養による再分化系統の推定染色体数と主要形質との相関関係

Table 2. Coefficient of correlations between estimated chromosome number and agronomic characters in clonal lines of sugarcane regenerated from leaf-induced calli irradiated with gamma rays.

形質	Character	相関係数 Correlation (r)
推定染色体数：葉長	Est. chromosome no. vs. Leaf length	+0.555**
〃：葉幅	〃 vs. Leaf width	+0.589**
〃：原料茎長	〃 vs. Millable stalk length	+0.552**
〃：茎径	〃 vs. Stalk diameter	+0.443**
〃：節数	〃 vs. No. of nodes	+0.210*
〃：原料茎重	〃 vs. Millabl stalk yiel	+0.541**
〃：糖度	〃 vs. Sugar content	−0.395**
〃：糖量	〃 vs. Sugar yield	+0.396**

注）Remarks：供試数 (n = 111)，有意水準 Significance: * 5%, ** 1%

Association among estimated chromosome numbers and agronomic characteristics of regenerated mutant lines derived from acute gamma-irradiated calli in sugarcane

In radiation breeding using *in vitro* culture, irradiation method often remarkably changes the characteristics of regenerated plants and lines. Hence, an effective method should be established based on an investigation of the association between irradiation method and characteristics of the regenerated lines. The association of irradiation dose and agronomic characteristics has been analyzed in the regenerated mutant lines derived from acutely irradiated calli in sugarcane. Nuclear DNA content has closely associated with chromosome number, and is considered as a measure to analyze change of chromosome due to irradiation damage in the lines.

Method: Using the sugarcane variety, Ni 1, calli induced from rolled leaves growing at a nearby point were exposed to acute gamma rays ranging from 50 to 500 Gy in terms of total dose for 20 hours. Soon after irradiation, the calli were transferred to new media, and regenerated plants were then established and transplanted in a field nursery. The yield and quality characteristics of 111 clonal lines from each regenerated plant were investigated and compared to the original ones. The relative nuclear DNA content of each line was measured on a leaf sample of a line mixed with one of the control, in which the chromosome number was known (Variety: Badila, 2n = 80) using flow cytometry (Partec). Furthermore, the estimated chromosome number of each line was calculated based on the ratio of nuclear DNA content to the control.

Results: A sugarcane cultivar is essentially highly polyploidy, aneuploidy and chromosome mozaicism, in which different chromosome numbers appear in each somatic cell. Therefore, the exact chromosome number of a cultivar is hard to count. The chromosome number of the original variety, Ni 1, is estimated to be about 2n = 116. There is no difference in the estimated chromosome number between the original and the 0 Gy groups; however, the estimated chromosome number of the irradiated groups was evidently decreased as the irradiation doses rose (Table 1).

A significant negative correlation was found between the irradiation dose and the estimated chromosome number in the regenerated lines, and the regression analysis between each characteristic showed that 3.3 chromosomes were lost at every 100 Gy in terms of the total dose of acute irradiation. At 500 Gy irradiation, which is a critical dosage for survival, 16 chromosomes of 116 were lost in the regenerated plants.

The growth of regenerated plants was retarded as the irradiation dose rose (Fig. 1). Highly positive correlations were found between the estimated chromosome number and the size of the leaf and stalk and yield of the stalk, while a negative correlation was detected between the estimated chromosome number and sugar content (Table 1). Eventually, regenerated lines decreased in chromosome as a result of acute irradiation and were also generally retarded in biomass production.

On the other hand, the range of variability of agronomic characteristics was narrow in 0 Gy, wide in 50 and 100 Gy, moderate in 200 Gy, and narrowly shifted to a lower level in 300 Gy and over. More vigorous lines than the original appeared in 50 to 200 Gy, showing that appropriate irradiation resulted partly in increased variability in the mutant lines.

In conclusion, optimum irradiation should reduce radiation damage, decrease any loss in chromosome, and enlarge variability in the yield, sugar content, and morphology of mutant lines; mutants with high yield and high quality could then be induced with a high probability.

(Shigeki Nagatomi and Konosuke Degi)

〒319-2293　茨城県那珂郡大宮町私書箱３号
独立行政法人　農業生物資源研究所　放射線育種場
Institute of Radiation Breeding, NIAS
P.O. Box 3, Ohmiya-machi, Ibaraki, JAPAN 319-2293