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バナナの倍数性突然変異の誘発および判別方法の開発

Development of polyploid induction and discrimination methods in banana

バナナは不結実性で，種苗の増殖率が低く，また巨大植物で広大な栽培面積を要するなど，通常の育種方法では改良が困難であった。しかし増殖率の高い培養系を用いた放射線育種法を開発し，その有力な改良手段となっている（テクニカルニュース No. 55）。さらに，放射線育種場では沖縄県農業試験場と共同でバナナの倍数体の誘発法および簡易検出法を開発した。

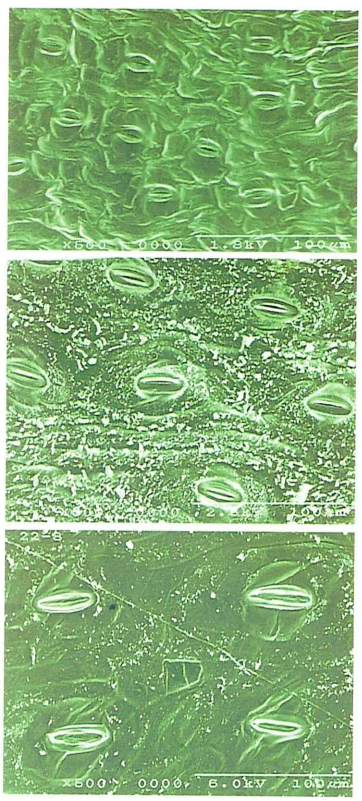
バナナの栽培種 *Musa* sp. (AAA genomes) を供試して，茎部柔組織を外植片としてカルスを誘導し，液体培養に移し100～500ppm 濃度のコルヒチン処理を行なった。一部のカルスは処理前に，ガンマ線による10，20Gy の急照射を行なった。処理カルスは3代の継代後、得られた再分化個体は温室で順化された。

順化後4カ月目に268個体を対象に生育および葉裏の気孔など6形質を測定し，相関マトリックスから主成分分析を行った。走査型電顕による葉裏の画像データから気孔の測定を行なった。倍数性の検定には，再分化個体の根端を採取し定法により染色体数を測定し，またフローサイトメータによる葉身の細胞核のサイズを測定した。

二倍性のバショウは気孔が最も小さく，三倍体の原品種はそれに次ぎ，作出した六倍体ではいずれも気孔が極大となった（第1図）。

個体群の茎長，葉長，葉幅の相互間に，また気孔数，葉長，葉幅の相互間には共に高い正の相関があり，一方，気孔長と茎長，葉長，葉幅，気孔数との間には負の相関関係が認められた。気孔長：y と気孔数：x との回帰分析を行った結果，有意な負の相関関係(r＝－0. 841)があり，$y=0.0321x^2-2.992x+134.045$の回帰式が求められた。この2形質による分布図では，3倍性と6倍性の個体がそれぞれ類別された。

主成分分析の結果，主成分1は茎長，葉長，葉幅，気孔数が正に，気孔長が負に寄与する植物体の生長量を示す”Size factor”であった。主成分2は気孔長，葉幅，葉長が正で，気孔数が負に関係する倍数性を表す成分であり，(＋)方向で6倍性を，また(－)方向で3倍性を表す“Shape factor”であった。主成分1と2との散布図から，6倍性と3倍性の2群を類別することができた(第3図)。6倍性群は3倍性群に比べて気孔が大となり気孔数は減少し，葉身が重厚で幅広い点に共通した特徴があった(第2図)。



第1図　バナナの二倍体，三倍体と六倍体(上より)の葉裏気孔

Fig. 1.　Stomata on abaxial leaf surface in diploid, triploid and hexaploid of banana.

第2図　バナナの3倍性（右）および6倍性（左）個体の草姿

Fig. 2.　Plants in triploid (right) and hexaploid (left) of banana.

高次の倍数性個体を生育初期に簡易に選別するには，葉裏の気孔サイズと葉身の核サイズから判定する方法である。沖縄ではバナナの低茎や果実サイズの突然変異体を選抜目標にして，倍数化による変異性の検定を実施中である。

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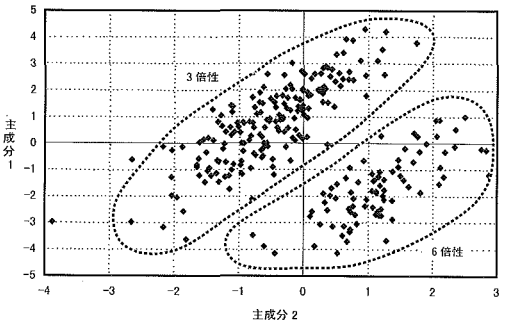

第3図　倍数剤処理再分化個体群の主成分1と2による散布図
Fig. 3.　Scatter diagram between principal component 1 and 2 for regenerated plants treated with colchicine in banana.

Development of polyploid induction and discrimination methods in banana

It is difficult to breed bananas for three main reasons: female infertility, very low propagation ability, and a very big plant. However, effective mutation breeding has been developed by using tissue culture techniques combined with mutagenesis. The Institute of Radiation Breeding, in cooperation with Okinawa Prefecture, has developed an effective induction method for polyploidy mutation and the discrimination methods in bananas.

Using a banana cultivar, *Musa* sp. (AAA Genomes), calluses were induced through explants of stem top tissues and transferred to the liquid basal media as shown in Technical News No. 55. The callus was treated with 100 to 500ppm colchicine for certain periods in liquid media then transferred to new liquid media. In advance, some calluses were irradiated at a total dose of 10 and 20Gy by gamma ray radiation. After three successive cultures, regenerated plants obtained were acclimatized in a greenhouse.

A total of 268 four-month-old regenerated plants were investigated, and six characteristics of growth habits and stomata were recorded. Principal Component Analysis (PCA) was performed based upon the correlation matrix. The stomata were investigated by scanning electron microscope, and chromosomes were counted in the root tip segments under an optical microscope.

The investigation revealed that the stomata were the smallest in the diploid variety, "Bashoo," followed by the triploid cultivar, and largest in the hexaploid mutant (Fig. 1).

Highly positive correlations were found among stalk length, leaf length and leaf width, as well as among stomata density, leaf length and leaf width. In contrast, stomata length was negatively correlated with stalk length, leaf length, leaf width and stomata density. Regression analysis between stomata length (y) and stomata density (x) revealed a highly significant negative correlation ($r=-0.841$) and yielded the equation $y=0.0321x^2-2.992x+134.045$. The scatter diagram of both characteristics segregated the regenerated plants indicated triploid and hexaploid groups.

PCA revealed that the first component was positively related with stalk length, leaf length, leaf width and stomata density, and negatively so with stomata length. Furthermore, its biological meaning was proven to be growth of the plant, or "size factor." The second component was positively related with stomata length, leaf width and leaf length, and negatively so with stomata density; its meaning was ploidy level, "shape factor," indicating hexaploid (+) and triploid (−).

The scatter diagram of the first and second components indicated two groups (Fig. 2). The characteristics of a hexaploid plant compared with the original triploid plant shifted towards larger stomata, lower stomata density and thick/wider leaf (Fig. 3).

In conclusion, an effective yet simple technique for selecting higher ploidy mutants at an earlier stage of banana growth is discrimination by stomata size and density on the abaxial surface. Further investigation is in progress to select desired mutants having less stalk and larger fruits in Okinawa.

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