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Effectiveness of gamma ray irradiation and ethyl methane sulphonate on *in vitro* mutagenesis of strawberry

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The experiment was conducted to study the effect of gamma-ray irradiation on the high concentration thidiazuron (TDZ) produced buds. *In vitro* buds were irradiated with different gamma-ray doses. Akihime cultivar ('Akihime') was irradiated with the doses of 0, 30, 80, 130, 180, and 230 Gy while 'DNKW001 accession' ('DNKW001') was exposed to the doses of 0, 30, 80, 130, 180, 230, 280, 300 and 325 Gy and similar doses of gamma rays + EMS 7μM (GRE) treatments. Survival rate and plantlet performance of DNKW001 in gamma ray + EMS 7μM treatment declined profoundly with increasing doses and LD_{50} was lower (104 Gy) than LD_{50} in gamma ray irradiation (177 Gy) alone. Variants of plantlets were detected in pre (white streaked leaf and bigger petiole with distorted leaf) and post acclimations (dwarf, dwarf-necrosis, variegated, dark-rigid-thick leaf, rumpled leaf, heart shape-bright red fruit). Hexadecaploid of Akihime and pentadecaploid, 13x + 4 chormosome, and diplodecaploid of DNKW001 were discovered sturdy plants with thicker leaf and bigger pollen than octoploid plant, while the monosomic octoploid performed a dwarf plant. Outstanding variants based on fruit weight, total soluble solid content and color in plot of Principle Component Analysis (PCA) were selected and proved as mutants in DNA level. Gamma ray irradiation + EMS was more effective to generate more type and magnitude of variants. Irradiation dose less than 130 Gy was ample for generating variant plants of strawberry.

Key words: Survival rate, LD_{50}, dwarf, thick leaf, monosomic octoploid, hexadecaploid, diplodecaploid, PCA

INTRODUCTION

Cultivated strawberry is an octoploid (2n=8x=56) and the complicated genetic background presents a formidable barrier mutation breeding. Genetic background of strawberry was composed by a few nuclear and cytoplasmic germplasm (Dale and Sjulin, 1990). Some efforts have been made to increase genetic diversity through somaclonal variation, mutation and cross breeding with wild relatives.

Somaclonal variation, like mutation breeding, can be valuable based on random variation. Exploitation of somaclonal variation has been carried out in chrysanthemum (Minano et al., 2009) and strawberry (Popescu et al., 1997; Sansavini et al., 1990; Biswas et al., 2009). The magnitude of somaclonal or abnormal plants depends on concentrations of plant growth regulator (PGR) (Anderson et al., 1982) and good variants were also obtained in strawberry (Biswas et al., 2009) even though in low concentration of PGR.

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Application of mutagen was intended to increase genetic variation and can change only one or a few specific traits of an elite cultivar that can contribute to fruit improvement (Predieri, 2001). In fruit crops, mutagenesis has already been used to introduce many useful traits affecting plant size, blooming time and fruit ripening, fruit color, better quality, self compatibility, self-thinning, and resistance to pathogens (Maluszynski et al., 1995; Kaushal et al., 2004). Gamma rays have been used in broad species of plants (rice, barley, cotton, groundnut, pulses, sunflower, rapeseed and pear, etc.) and developed 64% of the radiation-induced mutant varieties (Ahloowalia et al., 2004). Frequency of variants depends on genotype and dose of mutagen (Weimin et al., 2009). In strawberry, wide range (5-800 Gy) of gamma rays has been applied by researchers in different plant materials such as anther calli (Kasumi, 2002), calli of leaves (Kaushal et al., 2004), axillary bud (Jain, 1997), and runner (Weimin et al., 2009). Most of them applied gamma rays for inducing mutant in low concentration of PGR. Variants with different phenotypic characters were observed (Jain, 1997; Kasumi, 2002; Kaushal et al., 2004).

Another mutagen, ethyl methane sulphonate (EMS), also has been applied in various plants, such as soybean (Patil et al., 2007) and tobacco (Julio et al., 2008). Treatment of EMS in high concentrations as well as the combined treatment of both the mutagens c-radiation and EMS was effective in increasing the variability of fatty acid content in soybean oil (Patil et al., 2007) and produced homozygous M2 plants carrying nonsense alleles of NtabCYP82E4 that coded very low or near-null nornicotine (Julio et al., 2008). Comprehensive approach in producing variants by applying mutagens of gamma rays and gamma rays combined with ethyl methane sulphonate (EMS) on buds of explants produced in high concentration of PGR was elaborated in this work.

MATERIALS AND METHODS

'Akihime' and 'DNKW001' were used as plant materials for mutation induction. Mother plants were grown in the plastic house with standard cultivation and sprayed fungicide every 1 to 2 weeks. Tissue culture procedure followed the method of Yonghua et al. (2005) with slight modification. Young folded leaves were collected in the morning from health mother plant. Leaves were soaked in the detergent solution for 2 to 3 min, washed in tap water for 5 min and sterilized in clean bench with 70% (v/v) ethanol for 1 min, treated with 0.1% (w/v) $HgCl_2$ for 10 min, followed by rinsing three times with distilled water. Leaves were dissected aseptically to the size 2 to 3 mm^2. The explants were placed abaxial side down on the shoot regeneration medium.

Medium and culture condition

Explants were cultured on MS media containing 3% (w/v) sucrose, 0.8% (w/v) agar and 40.9 μM TDZ+IBA 2.5 μM (Murti and Yeoung, 2010). The pH was adjusted to 5.7 before adding agar and autoclaving (121°C, 15 min). Around 20 explants were put into each glass jar (∅ 8 cm) containing 30 ml medium. Explants were cultured in the dark room for 10 days at 27°C before placed under 2000 lx irradiance with 16/8 h day/night photoperiod in 23 ± 1°C. Plantlets were sub-cultured every 4 weeks in the proliferation medium with similar PGR and 6.8 μM BA, respectively. Doses of gamma rays that consisted of 30, 80, 130, 180, 230, 280, 300 and 325 Gy alone and combined with 7 μM EMS were applied to 'DNKW001', while 'Akihime' was irradiated with 30, 80, 130, 180, 230 Gy at 8 weeks after plating explants in medium. Each treatment consisted of 60 explants placed in triplicate. Gamma ray irradiation was applied in Korea Atomic Energy Research Institute (KAERI)-Advanced Radiation Technology Institute (ARTI), Jeongeup, Jeollabukdo Province, South Korea. Eight weeks old plantlets were exposed to Gamma rays (GR) irradiation for 24 h in different distance from irradiation source according to irradiation doses.

Sterile EMS 7 μM was applied four days after gamma rays treatment to accomplish the combination treatment of gamma rays and EMS 7 μM (GRE). Plantlets were soaked in the EMS solution for 1 h and rinsed with 7 μM of sodium thiosulphate. Plantlets were stored in the dark room for one week after each treatment for recovery their growth. The elongated plantlets were separated individually and cultured for 4 to 6 weeks on the basal medium without plant growth regulators for root initiation. Rooted shoots were taken out from rooting medium 4 to 6 weeks after rooting and were rinsed free of tissue culture medium, then grown in plug trays containing sterile 'Plant World' commercial media (Nongwoo Bio, Korea). Trays were placed in transparent closed humid chamber and acclimatized by gradually lowering humidity, with spraying fungicide regularly to prevent fungal infection. After 15 days, plantlets were moved to the green house for about one month which included under screen shading (60%) for 5 to 7 days and then fully exposed to sun shine.

The surface of the bench filled with 'Plant World' commercial media (Nongwoo Bio, Korea) was covered with white mulch. Alternate planting holes were made with 20 cm distance in row and 25 cm between rows. Automatic nutrient solution system was set up with drip irrigation system for water supply.

Chromosome observation

Chromosome observation was carried out with the method described by Preeda et al. (2007) with slight modification. The root tips of 3 to 5 variant daughter plants were collected separately in the evening (6.00 to 7.00 p.m.), pre-treated with 0.002 M 8-hydroxyquinoline for 1 h at room temperature, and subsequently kept at 4°C for 15 h. The roots tips were then fixed in carnoy II solution for 40 min at room temperature. The root tips were softened by hydrolyzing in 1N HCl at room temperature for 1.5 H and at 60°C for 10 min and then digested using the enzyme mixture of 4% cellulase (Sigma-Aldrich), 1% pectolyase Y-23 (Seishin Pharmaceutical Co. Ltd., Tokyo), 2.1% macerozyme R10, and 0.0075 M Na_2EDTA, pH 4.2 at 42°C for 40 min. After that, they were shortly-rinsed in distilled water. Root tips were placed on slide glass with a few drops of acetic acid (AA) 60% (v/v in ethyl alcohol) solution, and then stored in refrigerator at -20°C for 2 to 5 min. Samples were laid by tapping with fine forceps into invisible particles using a fresh drop of AA 60% and air-dried under room temperature. Air-dried specimens were stained in the 4% Gymsa solution diluted with 1/15 M phosphate buffer (pH 6.8) for 3 to 5 min and then warmed using an alcohol lamp for a few seconds.

RAPD procedure of Doyle and Doyle (1990) with slight modification in the steps of PCR reaction: predenaturation at 94°C (5 min), denaturation at 94°C (45 s), annealing at 41°C (1 min), elongation at 72°C(1.5 min), final elongation 72°C (7 min) was used. A total of eight random 10-mer primers (OPS12, OPB01, OPA02, OPB08, OPJ17, OPA01, OPB04, OPB14) were used for the PCR reactions.

Data recording and analysis

Survival rate of explants was the percentage of explants produced plantlets. The number of explant produced plantlets was recorded 2 months after applying treatments. While strawberry plants were growing, the growth parameters such as flowering, crown and infloresences number, fruit weight per plant, total soluble solid and fruit color of two fruit samples from each plant were investigated. Flowering date was determined when first flower of first infloresence fully opened, while recording of runnering plant was when the runner was 2 cm in length. Crown number and inflorescences were observed in five months after planting. The samples of root of three daughter plants in each intended variant were observed. Three to five of good spread chromosomes were used in chromosome counting. Fresh pollen was collected from greenhouse-grown normal and variant genotypes. Flowers were cut out and kept in the Petri dish to collect pollen. Pollen was placed on the microscope slide and stained with acetocarmine, and examined at magnify 40x with a high resolution dissecting microscope with illumination. For fruit characters: on two fruit, two external (on opposing shoulders) color measurements (Model CR-400 Chroma Meter, Minolta Camera Co., Ltd., Ramsey, NJ) were taken using the L*a*b* color space. Soluble solids content (%) was measured in duplicate with a digital refractometer (Model PR-101, Atago Co., Ltd., Tokyo, Japan).

Linear or quadratic equation of survival rate data were estimated for determining lethal dosage of Gamma ray (singly or combined with EMS) in DNKW001, but it did not apply to 'Akihime' because of unreliable data. Principle component analysis (PCA) was used to simplify the data structure and still accounted for as much of the total variation in the original data as possible and was used for selection. Analysis of variance and principle component analysis (PCA) of quantitative data were performed with SAS version 9.1, while qualitative data was descriptive.

RESULTS AND DISCUSSIONS

The young folded leaves were used as explants for generating plantlets. Using high concentration of TDZ (40.9 μM) with IBA (2.5 μM) was intended to spur the organogenesis; in spite of the low concentration, could produce plantlets in many genotypes (Landi and Mezzetti, 2006). Plantlets generated through direct organogenesis in high concentration of PGR were expected to produce more variation; although Biswas et al. (2009) produced more variants through somatic embryogenesis than direct organogenesis. 'DNKW001' accession conferred higher regeneration capacity in high concentration of TDZ (40.9 μM + 2.5 μM IBA) than 'Akihime' cultivar, as reported by Murti and Yeoung (2010).

Increasing doses of gamma rays decreased survival rate (percentage of explants produced plantlets) as shown in Figure 1. Survival rate and plantlets performance declined with increasing mutagens; similarly with irradiated calli in Kasumi (2002). Decreasing survival rate of plantlets in the combination of gamma ray irradiation and EMS treatment was higher than gamma rays irradiation alone. Kausal et al. (2004) found out that none lethal calli was shown until 50 Gy, but in this experiment lethal effect began at 30 Gy. The survival rate decreased markedly when explants were exposed to gamma rays more than 180 Gy, and even the survival rate achieved 4.4 and 2.9% and produced total plantlet number at the end of culture less than 10 plantlets at doses 300 and 230 Gy in Gamma ray and Gamma ray + EMS 7μM, respectively. This differed with the results of Kasumi (2002) which shows that the moderate decreasing plantlet started at irradiation dose more than 400 Gy. In contrary, at an extreme condition in potato, no emergence occurred at 60 Gy (Cheng et al., 2010). These different responses were caused by different irradiation models. Kasumi (2002) used an irradiation model of 10 Gy per hour, and the plantlet received pressure slowly to reach a certain dosage, while in this experiment, the irradiation was done at a short time of 24 h for all doses.

The pressure of gamma ray and EMS mutagens consecutively, caused more disruption in the steady state of the physiological process and genetic expression /rearrangement, and consequently the LD_{50} of combined treatment (104 Gy) was lower than for gamma rays irradiation alone (177 Gy). LD_{50} in this experiment was higher than in the results of Weimin et al. (2009) showing that the LD_{50} of Akihime was in the range 30 to 50 Gy, in Shuo Feng cv. It was above 80 Gy, and for others it was between 50-80 Gy. LD_{50} and maximum dosage for strawberry were higher than for lotus (Arunyanart and Soontronyatara, 2002) and rose (Kim et al., 2006). Apparently, LD_{50} differed between cultivars and species.

Combination of Gamma ray and EMS decreased the plantlets performance (data not shown), similarly in root number and shoot length of rose (Kim et al., 2006) and in leave number, leaf diameter, root number and rhizome number of lotus (Arunyanart and Soontronyatara, 2002). The variant plants observed in the tissue culture were a chimera of the white streaked leaf of DNKW001 in the gamma rays (30 Gy). Variants of Akihime plantlets treated with 80 and 180 Gy had bigger petiole than normal plantlet with distorted leaf (the end of petiole folded). Chimera recovered before acclimatization, while new leave with normal growth replaced the big petiole and distorted leaf plantlets after acclimatization. That variation was probably an expression of the epigenetic activation of DNA elements (Kaeppler et al., 2000) or mutagen that affected the temporary steady state physiology of the plantlet.

Morphological variation increased in accordance with replenishment of Gamma rays doses if applied alone while the highest frequency (5. 2 to 5.8%) and type of variation was between 80 to 130 Gy when Gamma rays was combined with EMS 7μM (Table 1). The highest variation in Gamma rays + EMS corresponds to the LD_{50} of Gamma rays irradiation in tissue culture. Variant plants showing dwarf, and rumpled, rigid-thick-pubescent leaf (Figure 2A) with bigger pollen (Figure 3), chimera leaves (mottle), chlorosis-necrosis (2C-D) were identified. One variant with bright red-heart shape-smaller size fruit (Figure 2B) was found in 30 Gy + EMS treated plant with normal shoot performance. This variant produced the smaller fruit although was a normal plant. It was different with Hortynski et al. (1991) conclusion that the small-

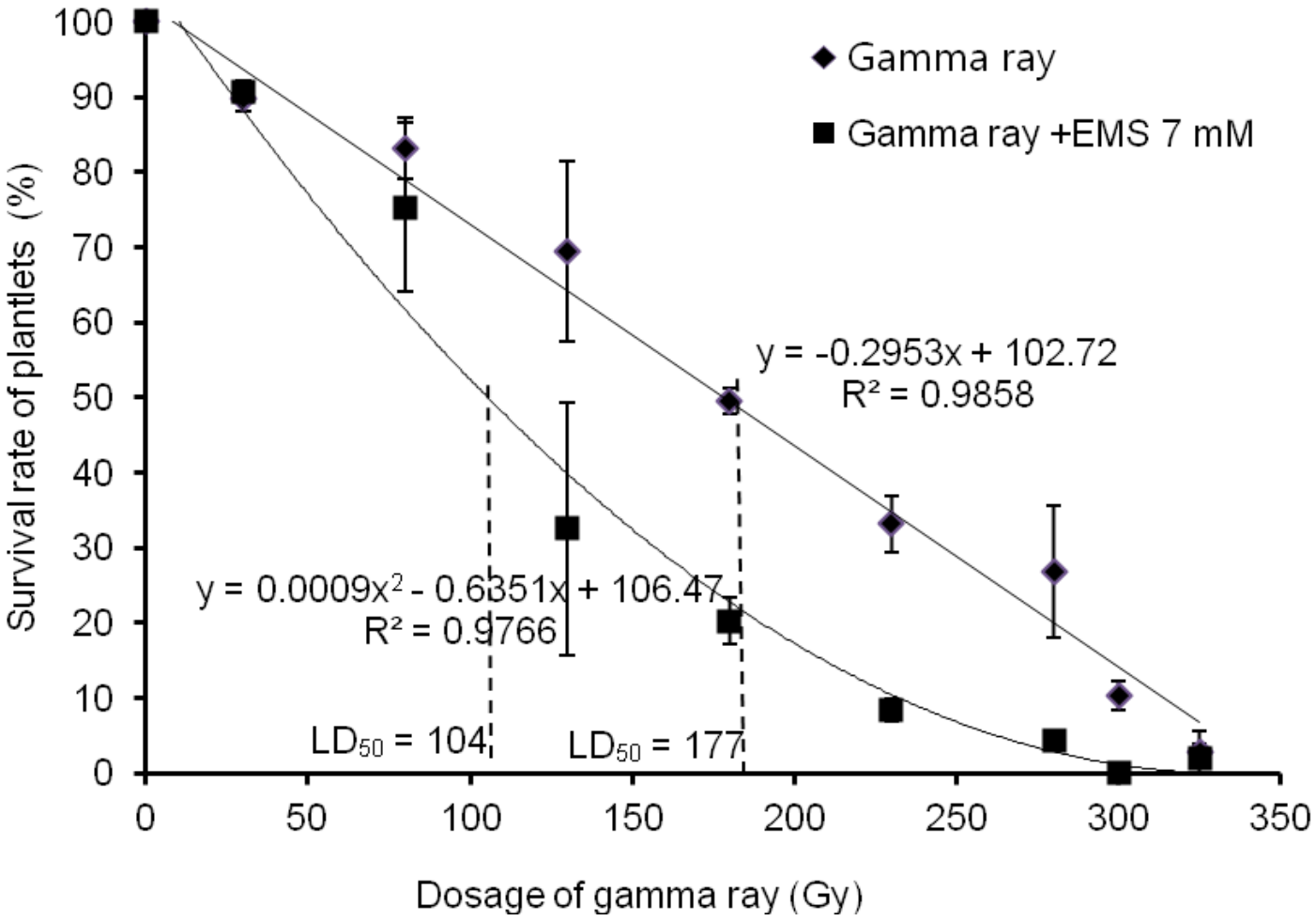

Figure 1. Effects of gamma ray and EMS treatment on the survival rate of 'DNKW001' strawberry plantlets.

Table 1. Percentage of morphological variant (%) in different doses of GR in 'Akihime' and 'DNKW001'.

Cultivar	Gamma rays doses (Gy)					
	0	30	80	130	180	230
Akihime	0.0	4.0	0.0	5.4	10.0	0.0
DNKW001	0.0	2.6	2.4	3.3	3.0	6.7
DNKW001 (+ 7 mM EMS)	0.0	3.5	5.2	5.8	4.9	0

fruited clones belonged to small leaves and a reduced photosynthetic area. This variant seemed to be promising mutant for cake accessory because of the smaller fruit size and longer shelf life (data not shown) than that of normal DNKW001.

Investigation of chromosome numbers of control and variants with thick leaf, smaller fruit-heart shape-red bright, and dwarf plants indicated the change of chromosome number (Figure 4). Variants of DNKW001 consisted of wild type/ normal plants (8x=56 chromosome), small-bright red fruit plant (8x = 56), dwarf (8x-1 = 55), thick leaf plants (diplodecaploid = 12x = 84, 14 x-3 = 95, pentadecaploid = 15x =105), while the thick leaf variants of Akihime had 112 chromosome (hexadecaploid). This finding is in accordance with that of Kuksova et al. (1997) that showed the somatic embryogenesis induced by gamma-rays lead to tetraploid variants. Higher ploidy level plants, clearly discernible from the normal (octoploid) plants, with thicker and less numerous leaves, shorter and thicker petioles were same with result of Simon et al. (1987).

Double chromosome number of octoploid suggested that high concentration of TDZ and gamma ray irradiation simultanously could induce the chromosome doubling by

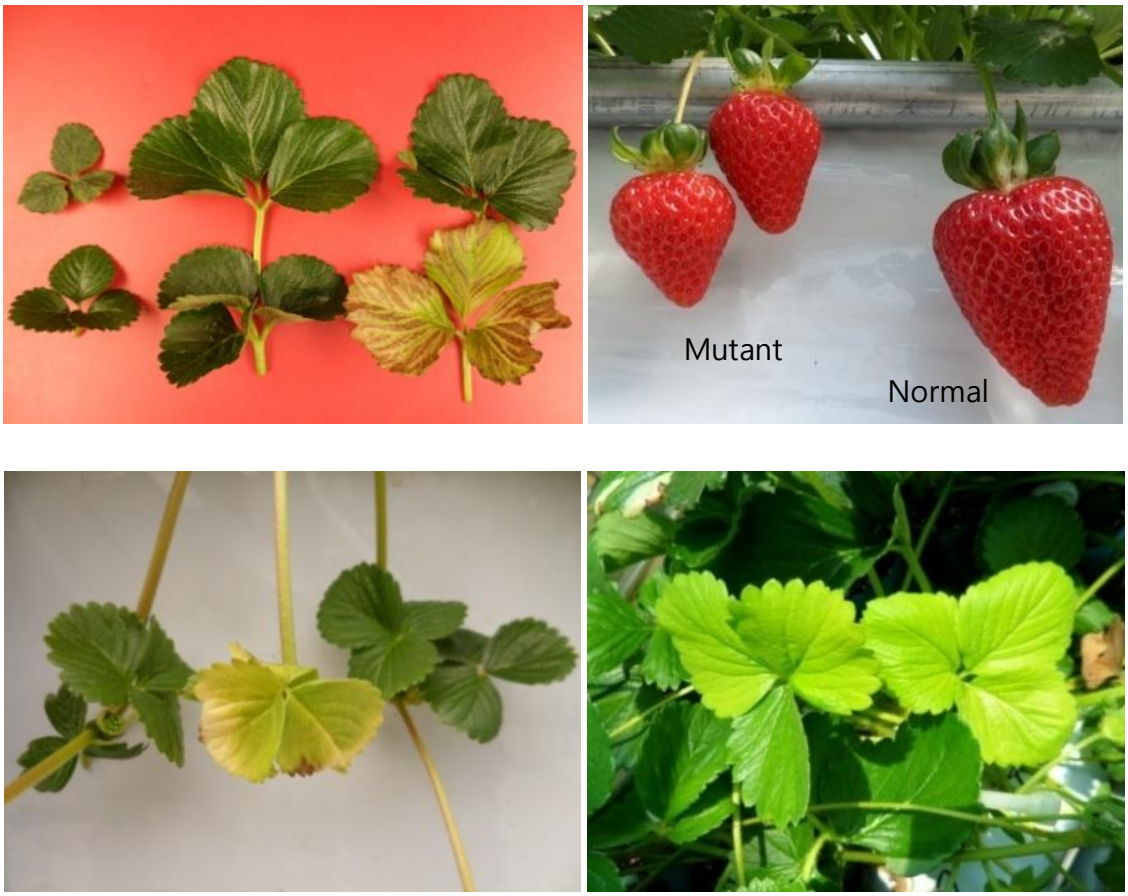

Figure 2. Above: Leaf variants from top left: dwarf-variegate, normal, scratch epidermis, dwarf, thick, and yellow leaf (left) and a variant with light red-heart shape-smaller fruit (right); **Below**: variegate and yellow leaves (left) produced by different crown in a variant plants with three crown: yellow leaf, variegate, and green (right).

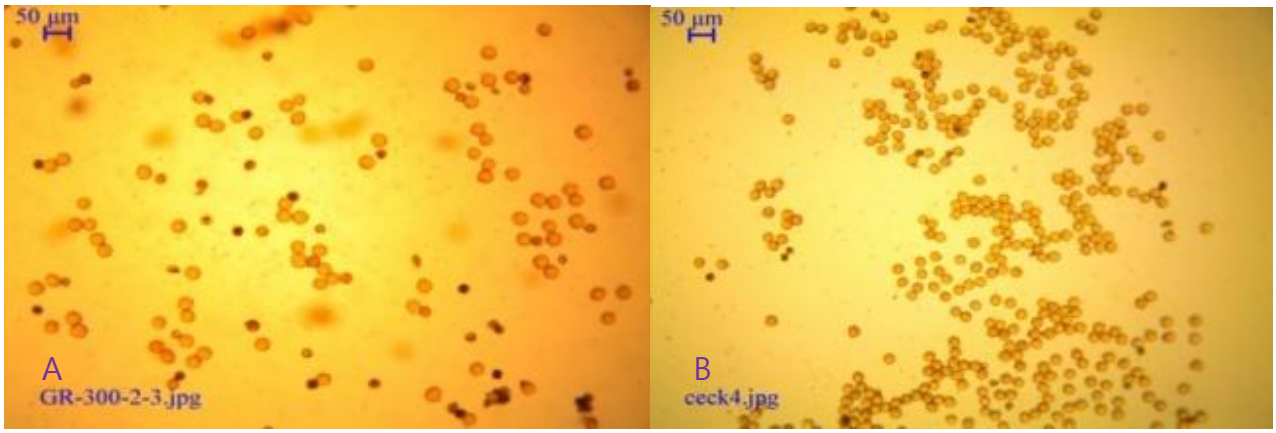

Figure 3. Pollens of thick leaf variant (A) and normal (B) plant.

counteracting the spindle at mitosis division of chromosome. Chromosome doubling was result of arrested cell division by the gamma-irradiation as found in suspension culture of *Ephorbia characias* by Chagvardieff et al. (1989), single or repeated rounds of endoreduplication (duplication without mitosis) (Kondorosi et al., 2000) or repetition of the S period known as endomitosis or reduplication (Yu, 1966). Another mechanism was caused by monopolar spindle (only a single spindle pole forms) then produced a single daughter cell with its copy number dou-

DNKW001:

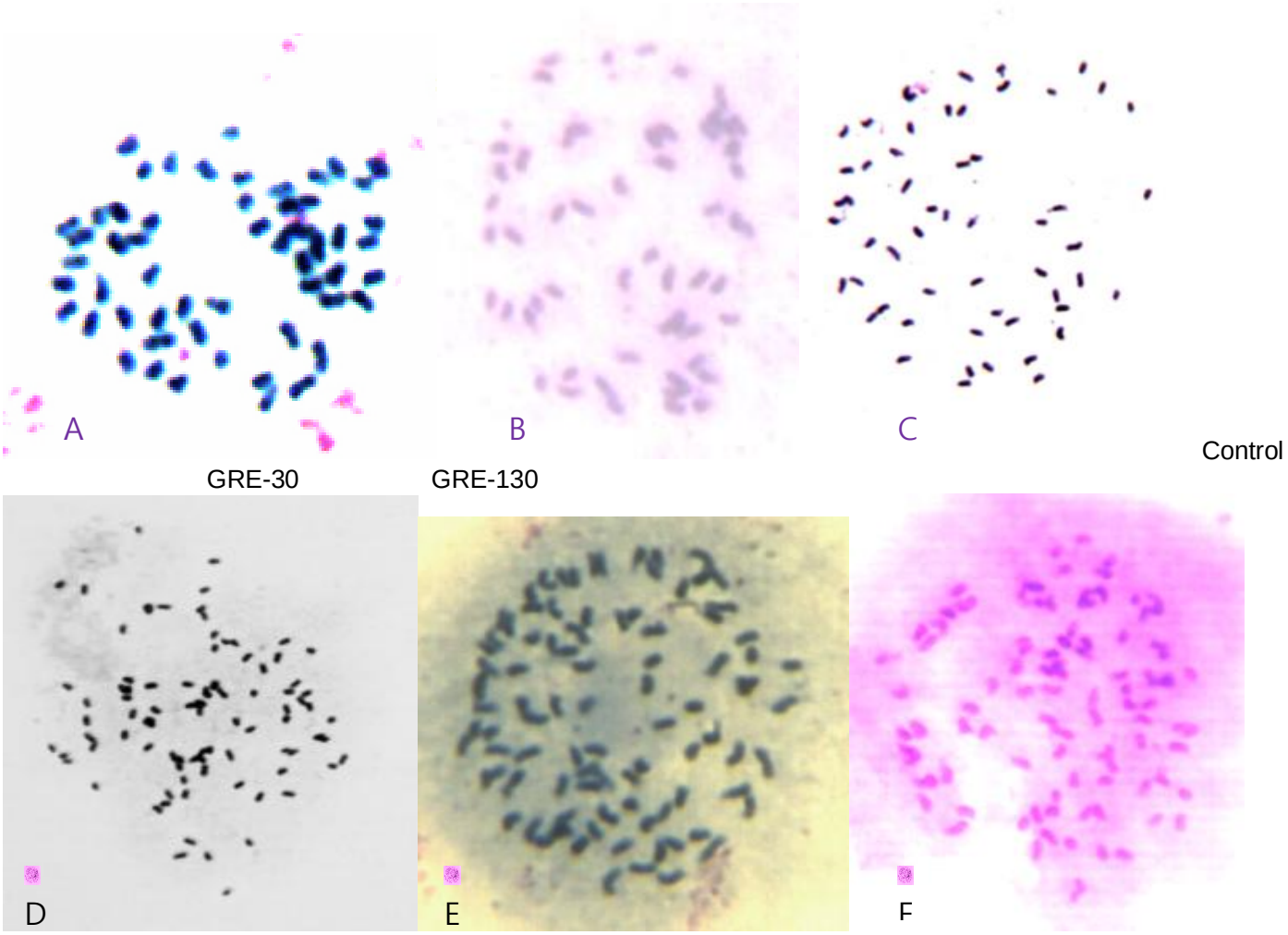

Figure 4. Chromosome of DNKW001: A = normal (8x=56 ch), B = small-bright red fruit (56), C = dwarf (8x-1=55), D-F = thick leaves (12x=84, 14x-3=95, 15x =105); Akihime: A = control (56), B-C = thick leaves (112).

doubled. Such cells have been known to contain more or twice the amount of DNA, RNA, etc. than normal cell

Table 2. Coefficients and eigenvalues from the first three principle component analysis based on Pearson correlation matrix between fruit weight/plant, brightness and total soluble solid of DNKW001 and Akihime cv. treated by Gamma rays and EMS.

Cultivar	Character	PC1	PC2	PC3
	Fruit weight/plant	0.61	-0.46	0.64
Akihime cv. (GR)	Brightness	0.44	0.87	0.21
	Total soluble solid	0.65	-0.15	-0.74
	Eigenvalues	1.25	0.94	0.79
	Percent of total variance	42%	32%	26%
	Fruit weight/plant	-0.52	0.72	0.44
DNKW001 (GR)	Brightness	0.54	0.68	-0.48
	Total soluble solid	0.65	0.01	0.75
	Eigenvalues	1.32	0.92	0.75
	Percent of total variance	44%	30%	26%
	Fruit weight/plant	0.49	0.82	0.28
DNKW001 (GR + E)	Brightness	0.58	-0.55	0.59
	Total soluble solid	0.64	-0.15	-0.75
	Eigenvalues	1.37	0.89	0.72
	Percent of total variance	45%	30%	25%

GR = Gamma rays; E = 7 μM EMS.

(Richard and Atkin, 1959) and it could be inherited to the progenies (Kondorosi et al., 2000). The process of others ploidy did not explainable yet.

In this experiment, the chimera plants with normal growth was produced in 80 Gy + EMS and two dwarf plants resulted in DNKW001 with 80 Gy and another in Akihime (180 Gy) but died before that produced runner. Similar result was found by Sansavini et al. (1990). In this experiment, the dwarf plant loss a chromosome, subsequently it had 55 chromosome (8n-1 or monosomics octoploid). It was allegedly the gene of of indole-3-acetic acid (IAA) and IBA that control the plant growth; attached in the losing chromosome. In Olmo (1934) studies, the monosomics (2n-1) tabacum plants exhibited reduced vigor, fertility and survivability. Another reason was mutation of the gene controlling plant growth regulator. An evident in cotton plants carrying the *sda* gene contained lower levels of IAA and abscisic acid (ABA) compared with wild-type (WT) plants. The chlorophyll content and net photosynthetic rate in mutant leaves were markedly decreased. However, it was possible that ABA biosynthesis or signaling was involved in governing the *sda* phenotype (Wu et al., 2009). Pollen sizes of variants plant with thick leaf were bigger than the normal plants (Figure 3) in both Akihime and DNKW001. Thick leaf plants had bigger size and lower stainability (42.7 to 67%) of pollen than the control (84.6 to 94.5%). Similar case was found by Ragone (2001) in breadfruit in which triploid cultivars had the lowest pollen stainability, averaging from 7 to 16%, and the pollen grains were typically malformed, clumped, and poorly stained.

PCA result is summarized in Table 2 and Figure 5. In Akihime, cumulative proportion of first and third eigen value achieved 0.68 that means 68% of total variance was explained by first and third principle components. In the first principle component, all variable had positive coefficient, while positive coefficient was in fruit weight/plant and total soluble solid in the third principle component. The selected plants based on the graph with first and third principle component axis were GR130-11; produced 43 fruit/plant, 77% marketable fruit (>10 g/fruit) and TTS 9.8 brix, while GR230-7 produced 30 fruits/plant, 70% marketable fruits, and TTS 9.0 brix. While in GRE treatment of DNWK001, the outstanding plant was 80-E13, 80-E90, 80-E70 that produced fruit of 498.8, 425.6, 409.7 g/plant; fruit number was 20, 22, 19 fruit; and TSS was 8.9, 7.5., 8.7 brix, respectively. Almost all selected plants in Akihime were generated in high doses of Gamma

Table 3. RAPD analysis of selected plants of Akihime and DNKW001 with eight primers.

Primer	DNA	Akihime				DNKW001 Gamma ray + 7µM EMS					
	Size/	A	B	C	D	E	F	G	H	I	J
OPJ17	200	0	0	0	1	0	1	0	0	0	0
	300	0	1	0	0	0	0	0	0	0	0
	400	1	1	1	1	1	1	1	1	1	1
	500	1	1	1	1	1	1	1	1	1	1
	700	1	1	1	1	1	1	1	1	1	1
	1000	1	1	1	1	1	1	1	1	1	1
	1500	1	1	1	1	1	1	1	1	1	1
	1700	1	1	1	1	0	0	0	0	0	0
	2000	0	1	0	0	0	0	0	0	1	1
OPA01	300	1	1	1	1	1	1	1	1	1	1
	1200	1	0	1	1	0	0	0	0	0	0
OPB04	400	1	1	1	1	1	1	1	1	1	1
	800	1	1	1	0	1	1	1	1	1	1
	1000	1	1	1	1	1	1	1	1	0	0
	1500	1	1	1	1	1	1	1	1	0	0
	1600	0	0	0	0	0	0	0	0	1	1
	1900	0	0	0	0	0	0	0	0	1	1
OPB14	250	1	1	0	1	1	1	1	1	1	1
	800	1	1	0	1	1	1	1	1	1	0
	1500	0	0	0	0	0	0	0	1	0	0
	1600	1	1	1	1	1	1	1	0	1	1
	2000	1	1	0	1	1	1	0	1	1	0
	2100	0	1	0	1	1	1	0	1	1	0

A = Akihime, B = Ca 0-1, C = Ca 130-11, D = Ca 230-7; E = DNKW001, F = GRE 30-2, G = GRE 80-13, H = GRE 80-70, I = GRE 80-90, J = GRE 130-13. There were no polymorphism in primer OPB01 (5 bands), OPA02 (5 bands), OPB08 (6 bands) and OPS12 (2 bands).

ray, while lower doses were appropirate to pro-duce selected plants in DNKW001. This result is in contrast to those of Weimin et al. (2009) in which Akihime cv. was the most sensitive cultivar to Gamma ray. RAPD analysis is most often expressed in a dominant fashion; it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). It is also possible that for a diploid dominant homozygote at a particular locus, mutations affecting only one of the two alleles would remain undetected (Isabel et al, 1993). In this study, two types of polymorphism appeared: fragment size differences and treatment of gamma rays irradiation and EMS was more effective than gamma rays irradiation that shown with lower LD_{50}, more frequency and type of mutation. The the absence of the fragment (null phenotype) (Table 3). High levels of sequence homology at many sites indicated by presence of RAPD marker between genotype in selected plants and wild type different polymorphisms were observed. There is the certainty of sequence difference when the marker was present in one genotype but not the other. The changing locus number in Akihime selected plants was two locus in average, and one to six loci occurred at DNKW001 selected plants. OPA01 produced one polymorphism, while nine polymorphism was produced by OPB14 in six selected plants in both cultivars DNKW001 and Akihime. In conclusions, combination changing of morphological characteristics of variant plants was as a result of genomic (euploidy and uneuploidy), while selected outstanding plants were affected by mutation

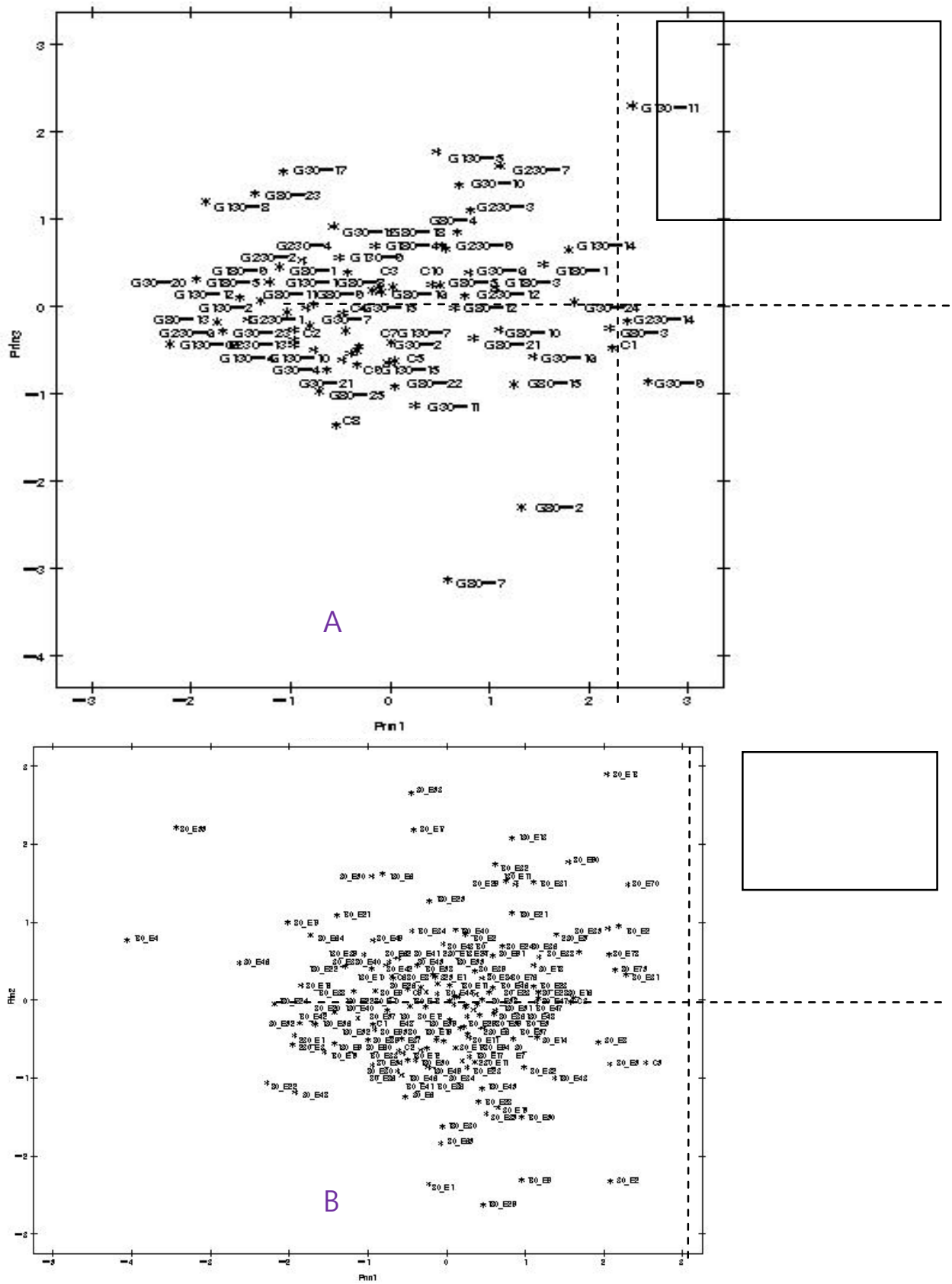

Figure 5. Plot of principle component analysis of Akihime (A) and DNKW001 (B) treated plants based on the firmness, total soluble solid and color.

in DNA level.

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