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Morphological and Anatomical Characters of Ploidy Mutants of Strawberry

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ABSTRACT

An experiment was conducted to elaborate the effect of different ploidy level on morpho-anatomical and physiological characters of strawberry. Wild type (8x=56 chromosome) and ploidy mutant plant of DNKW001 (diplodecaploid=12x, 4x-3=95, pentadecaploid=15x) and Akihime (heksadecaploid=16x) were used in this experiment. Morpho-anatomical, physiological, pollen characters and seediness were observed. Increasing ploidy level increased petiole stem diameter, leaf thickness, stomata size, plastid number per cell, SPAD value, chlorophyll content, and pollen size but decreased stomata density per unit area, pollen fertility, and no fruit set as result of seedlessness. There were no tendencies of different ploidy level of mutant on increasing magnitude of characters, except in pollen size and viability of DNKW001. Seediness could be improved by artificial pollination with octoploid pollen. The ability in germination of pollen and crossing of ploidy mutant with lower ploidy, excepted Ca 130-13, was an opportunity to use ploidy mutant in intercrossing specific traits of wild type into *Fragaria x ananassa*. © 2012 Friends Science Publishers

Key Words: Different ploidy; Leaf thickness; Plastid; Stomata; Pollen; Fertility; Seediness

INTRODUCTION

In previous experiment the obviously morphological variant strawberry plants of Akihime and DNKW001 were generated. These were confirmed with chromosome number such as hexadecaploid (16x), pentadecaploid (15x), 14x-3, diplodecaploid (12x) plants. All variant plants were produced through uncommon method like gamma ray irradiation on plantlets (Murti *et al.*, 2011). A polyploidy plant usually was produced through colchicine treatment (De & Maine, 1989).

The changing of morphological characters to be bigger size as consequent of polyploidy was well known (Simon *et al.*, 1987; Nyman & Wallin, 1993; Kulkarni & Borse, 2010), included the vegetative and reproductive organ (Butterfass, 1983). On another hand, the smaller or dwarf plant was affected by reducing chromosome number (Klahre *et al.*, 1998; Murti *et al.*, 2011). Polyploidy reflects the close general relationship between nuclear DNA content and cell size, and has interrelated effects on structural, biochemical, and physiological elements (Baer & Schrader, 1985; Warner *et al.*, 1987). Increased ploidy level series showed an increase in mean cell size in wheat (Dean & Leech, 1982), several cellular and chloroplast features in mesophyll cells in cereals (Dean & Leech, 1982; Jellings & Leech, 1984; Pyke & Leech, 1987).

The photosynthetic rate per leaf area was highly correlated with the DNA content per leaf area (Warner & Edwards, 1989). Polyploidy often increased the chloroplast number, then it may influence photosynthetic rates Baer and Schrader (1985); Warner *et al.* (1987). Photosynthetic carbon uptake increased in higher ploidal levels on a leaf area basis, such as in *Agropyron cristatum* (Frank, 1980), *Festuca arundinacea* (Joseph *et al.*, 1981), and *Panicum virgatum* (Warner *et al.*, 1987). Otherwise, the number of photosynthetic cells per unit leaf area progressively decreased with increasing ploidy from diploid to hexaploid, but thereafter remained constant in octaploid and decaploid plants (Warner & Edwards, 1989; 1993).

Flowers of autotetraploid plants of *Euchera grossulariifolia* were larger, shaped differently with different flowering phenology than that of diploids, and attracted different suites of floral visitors (Segrave & Thompson, 1999). Generative organ such as pollen is very important in strawberry fruit production. The polyploid was characterized by larger pollen grains, but was lower than control in pollen fertility, fruit set and seediness as well (Ragone, 2001; Yuwei, 2006), characterized with typically malformed, clumped, and poorly stained (Ragone, 2001) and faster pollen viability drops (drop > 50% after 6 h) (Bots & Mariani, 2005). Cross-pollinations, to overcome the low fruit set and seediness, resulted in a higher fruit set of mandarin citrus (Vithanage, 1991).

There is no information on the effects of ploidy level on morpho-anatomical, and physiological characters of strawberry in vegetative and generative stage. This paper

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elaborates the effect of increasing ploidy level on morphological, anatomical and physiological characters of strawberry plant at vegetative stage and flower characters and seediness in generative stage.

MATERIALS AND METHODS

The experiment was conducted in the plastic house with manual windows in each side to adjust the temperature and humidity. Polyploid mutants (Table I) and octoploid (control) plants of Akihime and DNKW001, identified in previously (Murti *et al*., 2011), were used in this experiment. The identified mutant plants were kept to produce daughter plants. The different ploidy plants were arranged in completely randomized design with three replications. The daughter plants were grown in plastic pot (ϕ 8 cm) and filled with 'Plant World' commercial media (Nongwoo Bio, Korea). The established daughter plants were excised from mother plant and grown in the bigger pot with similar medium. Water-nutrient supply is automatic in every hour or once in thirty minute depending on the sunshine. Complete nutrient solution was supplied through drip irrigation system with emitters at 30 cm spacing and a flow capacity of 2 L min-1 per 30 m tube length, containing nutrient solution with the composition (in mg L^{-1}): KNO_3 (286), $Ca(NO_3)_2$•$4H_2O$ (354), $MgSO_4$•$7H_2O$ (123), KH_2PO_4 (91), NH_4NO_3 (7), H_3BO_3 (3), $MnSO_4$•$4H_2O$ (2), $CuSO_4$•$5H_2O$ (0.05), $ZnSO_4$•$7H_2O$ (0.22), $NaMoO_4$•$5H_2O$ (0.02) and EDTA-Fe, 20. The pH and EC of the solution was 5.6 and 1.0 dS m^{-1}.

Anatomical and physiological parameters: Pyke and Leech (1987) method was used in determining of chloroplast number in mesophyll. A part of three leaves of different plants from each genotype was harvested. From each leaf a 5-mm section was cut and sliced into approx, 2-3 mm^2 pieces and fixed in 3.5% (w/v) glutaraldehyde in a small stoppered vial in the dark and 4°C for 1 h with shaker. Sections were then placed in 0.1 M sodium ethylene-diamine-tetraacetate (NaEDTA) (pH 9) and shaken in a water bath at 60°C for 3 h. After cooling, all materials were stored at 4°C. Pieces of tissue were macerated on a slide in 0.1 M NaEDTA and viewed with a "Mikroskop Technik Rathenow" (Germany) microscope using Infinity1 differential interference optics. Individual mesophyll cells were quickly separated on maceration and all of the chloroplasts within each cell could be counted easily. Thus in the three leaves of each genotype, a total of 21 cells were counted.

Stomata density was observed as described by Yuwei *et al*. (2006) with slight modification. The epidermis strips were carefully peeled from the abaxial surface of the fully developed leaves with smearing of nail dye; then observed under microscope (magnified 40x; produced by Leica Microsystems GmbH, Wetzlar, Germany) equipped with a color Coolsnap digital camera (Roper Scientific, Tucson, AZ) and the MetaVue software (Universal Imaging Corporation, West Chester, PA). Ten replicate measurements of length and aperture area of stomata were determined in each object using the ImageJ software. The samples were collected from three sample plants for each genotype.

Chlorophyll content was determined by SPAD-502DL (Konica Minolta Sensing Inc., Osaka Japan). Three plants were recorded and ten reading in each plants. Photosynthetic rate was determined with LI-6400-40 portable photosynthesis system. This chamber accessory to LI-6400XT allows for simultaneous measurements of gas exchange and fluorescence over the same leaf area, while the leaf thickness, diameter of petiole and flower size (corolla) were determined with a digital venire caliper.

Generative parameters: For measuring pollen size and percentage of pollen viability, pollens of three flowers in second inflorescence of each genotype were collected. Fresh pollen was collected from un-opened flower from normal and ploidy mutant. Flowers were cut and kept in the Petri dish, in laboratory then pollens were fixed in the acetic acid ethyl alcohol (1:3 v/v) for 1 h and placed in 70% ethyl alcohol until used. Pollen was placed on the microscope slide and stained with acetocarmine, examined at magnification 10x with a high resolution dissecting microscope with illumination provided from below the slide. Yellow staining pollen was scored as fertile type and wrinkle-small-dark was scored as sterile (unviable) type. Pollen size was determined according to standard bar with ImageJ software, while pollen germination observation was adopted from Wang *et al.* (2003). Opened flower (dehiscence of pollen) were collected. Stored pollen grains were equilibrated at room temperature for 30 min and suspended in 12% (w/v) sucrose medium containing 0.01% (w/v) H_3BO_3 to a concentration of no more than 30 pollen grains per 0.02 mL of medium. The cultures were adjusted to pH 6.4 with 0.1 M HCl or 0.1 M NaOH and incubated on a shaker (100 rpm) at 25°C for 3 h. Germinated pollen was observed under microscope and ten image of each slide was captured.

Pollination: The flowers were pollinated naturally and artificially. Artificial pollination was carried out with following steps. Pollens of the octoploid plants were collected by shaking flowers and pollens were placed in the small container. Collected pollen was scrubbed gently onto receptive pistil of mutant plant by fine brush. Pollinated flower then was marked and observed the fruit set and seediness. Seediness was expressed in percentage to normal fruit of wild type. Ripen fruits, as result of artificial pollination, were harvested and skin fruit with seeds was sliced and then extracted in the mixer with enough water. The pithy seeds precipitated were counted.

Statistical analysis: Collected data were subjected to analysis of variance (ANOVA) using the SAS statistical software package, release 9.1. Statistical F-tests were evaluated at $P<0.05$. Differences between treatments were further analyzed with Duncan's multiple ranges tests.

RESULTS AND DISCUSSION

DNKW001 accession plants are substantially open and erect growth type. Leaves (including petioles) for DNKW001 are shorter than Akihime, mainly due to shorter petiole length. Results of means separation by Duncan's Multiple Range Test (DMRT 5%) showed significantly different morphological characters and anatomical characters between control and mutant plants (Table I). In this experiment, the effect of increasing ploidy level changed the performance such as shorter, sturdy and morphological characters such as in leaves and flower. Petiole diameter of Akihime (3.2 mm) was thinner than DNKW001 (3.8 mm), while petiole diameter of mutant plants was significantly higher than control plant in both cultivars. There were no significant differences in petiole diameter among mutant plants of DNKW001 but it did not occur in Akihime. The ploidy mutant had thicker petiole (leaf stalk) and lamina (leaf blade) in both cultivars. Rauf *et al.* (2006) also reported that tetraploid plants of arboretum (4x=2n=52) had larger vegetative parts. Similar characters were found in strawberry from protoplast culture (Nyman & Wallin, 1993), Mulberry (Chaicharon *et al.*, 1995), *Salvia miltiorrhiza* (Gao *et al*., 1996), *Arboretum* (Rauf, 2006), *Petunia* (Guo-gui *et al*., 2009), *Spathiphyllum wallisii* (Vanstechelman *et al*., 2009), *Spathiphyllum* (Laere *et al.*, 2010). There was significant difference between Akihime mutant in both parameters and leaf thickness in DNKW001, although both of them were hexaploid. The different mutation in gene levels may be involved or different numbers of gene were included. There was no trend of increasing ploidy level to thickness of petiole and leaf of mutant. The increment of thickness did not occur and allegedly would be constant after certain ploidy level. Similar case was noticed by Warner and Edwards (1989) and Warner and Edwards (1993) in other parameter (photosynthetic rate). The thickness of leaf was associated with greenness of leaf.

Chloroplast number per cell (Fig. 1) was counted after small pieces of leaf processed as aforementioned in methods. This number in wild type was less than mutant plants in both cultivars (Table I). Chloroplast arises from undifferentiated plastids in small mitotic cells and replicate during normal mesophyll cell development (Boffey *et al.*, 1979). Consequently, the chloroplast number increases during mesophyll cell expansion producing large population of chloroplast in each mature mesophyll cell. The result in this experiment showed that chloroplast number in mutant increased dramatically compared with wild type. In DNKW001, increasing number of chromosome up to 95 increased the chloroplast number, beyond which this number did not increasing with further increase in ploidy level. Increase in chloroplast number was correlated with cell size as previously noted by Ellis and Leech (1985). Pyke (1999) reported that in spite of variation in cell size, plastid size, plastid number, total chloroplast area in cells were strictly related with cell size over a 10 fold range of cell sizes from different species. However, Ellis and Leech (1985) found that the number of chloroplast in fully expanded mesophyll cells was positively correlated with plan area of the cell in *Triticum aestivum* and *T. monococcum.* They also concluded chloroplast population size is species specific and can be variety specific.

The chlorophyll meter or SPAD meter is a simple, portable diagnostic tool that measures the greenness or relative chlorophyll concentration of leaves (Cate & Perkin, 2003; Netto *et al.*, 2005; Naus *et al.*, 2010). Compared with the traditional destructive methods, the used of SPAD-502 saves time, space and resources (Netto *et al.*, 2005). The result of SPAD data in this experiment indicated that the wild type had significantly lower values than mutant of both cultivars. This implied that the increasing ploidy level make up higher photosynthetic pigment. There were significant differences between mutants but no tendency of chromosome number and SPAD value. Markwell *et al.* (1995) and Netto *et al.* (2005) constructed the equation [chlorophyll (µmol m^{-2})=10($^{M\wedge0.265}$)] of chlorophyll content and SPAD values with very high coefficient determinant. The estimate value was used for estimating chlorophyll content. The estimate of chlorophyll content was 646-771 µmol m^{-2} for Akihime and 691-770 µmol m^{-2} in DNKW001. The highest estimate Chl concentration was 771 and 770, in Ca 130-13 (112x) and GR 30-63 (95x). Mutant plants had higher chlorophyll content than wild type, which is in accordance with Ntuli and Zobolo (2008).

The decrease in stomata number was linearly with increasing of stomata size. Stomata size (Fig. 1) as characterized in length of stomata and aperture area of stomata increased by increment of ploidy level. Stomata number in control (wild type) was significantly greater than ploidy mutant type in both cultivars, Akihime and DNKW001 (Table I). In DNKW001, increasing ploidy level produced lower stomata number per unit leaf area and relatively constant before chromosome number completely double. But for Akihime, there was significant difference in stomata number in the similar ploidy level of mutant. Decreasing stomata number caused by increasing ploidy level has been observed in *Stevia reboudian* (De Oliveira *et al.*, 2004), *Salvia miltiorrhiza* (Gao *et al.*, 1996), *Spathiphyllum wallisii* (Vanstechelman *et al.*, 2009), *Tetraploid spathiphyllum* (Laere *et al.*, 2010), *Miscanthus sinensis* and *M. sacchariflorus* (Rayburn *et al.*, 2009). Rayburn *et al.* (2009) reported correlation of stomatal size with genome size in *M x giganteus*, *M. sinensis* and *M. sacchariflorus*. This study also showed that stomata size was affected by ploidy level, though it did not occur linearly until at highest ploidy level. Therefore the stomata size (Omidbaigi, 2010) and protoplast number of stomata cell guard (Winarto *et al.*, 2010) could be used to distinguish normal and polyploid plant.

The low number of stomata on ploidy mutant appears as a consequence of the increasing size of cell, with no

Table I: Morphological and anatomical characters of different ploidy plants

Clones	Chromosome number	Petiole diameter (mm)	Leaf thickness (mm)	Plastids (number/cell)	SPAD	Chlorophyll (mmol m^{-2})*	Stomata		
							Number (mm^{-2})	Length (μm)	Aperture area (μm^2)
Akihime	56x	3.2c±0.06	0.24c±0.008	22.9c±1.08	49.4c±0.31	646±17	105a±1.32	12.0b±0.78	43.1c±1.89
Ca130-13	112x	4.7a ±0.13	0.37a±0.010	49.7a±0.86	54.7a±0.60	771±15	55 b±2.0	18.3 a±1.66	86.1a±1.52
Ca30-3	112x	4.2b ±0.05	0.33b±0.013	46.2b±0.93	50.7b±0.83	677±19	75 b±3.1	12.6b±0.32	61.5b±1.53
DNKW001	56x	3.8b±0.21	0.28c±0.003	24.9c±1.50	51.3c±0.70	691±16	105 a±1.6	10.8b±1.19	36.6c±1.75
GRE80-25	84x	4.8a±0.24	0.34b±0.025	33.5b±0.25	53.7ab±0.35	747±8	47 c±0.6	16.1a±2.16	82.8a±1.43
GR30-63	95x	4.5a±0.23	0.45a±0.029	41.9a±2.13	54.6a±1.01	770±25	88 b±0.9	12.3b±1.37	56.6b±1.16
GRE80-2	95x	4.8a±0.15	0.36b±0.005	42.2a±0.72	52.7bc±1.48	723±36	52 c±2.0	16.9a±1.76	82.8a±1.39
GR80-3	105x	4.6a±0.06	0.45a±0.018	43.4a±0.69	53.9ab±0.38	754±9	52 c±0.6	17.5a±1.17	90.0a±1.23

Note: Numbers in column with the same letter are not significantly different by DMRT a=5%; * estimate with equation $Y=10^{(M^{\wedge}0.265)}$, M is SPAD value

Table II: Generative characters of ploidy mutant plants

Clones	Chromosome	Flower diameter (mm)	Pollen			Seeds per fruit (%)*
			Size (μm)	Viability (%)	Germination (%)	
Akihime	56	30.2b±1.08	23.3c±0.67	69.0a±12.4	44.9a±4.7	105
Ca 130-13	112	35.8a±1.26	35.8a±1.31	5.5c±0.40	5.3c±0.7	4 (3.8)
Ca 30-3	112	36.1a±1.37	28.1b±3.57	46.0b±8.99	17.3b±2.9	23 (21.9)
DNKW001	56	31.1c±1.89	22.5d±0.54	81.6a±4.09	26.5a±2.1	131
GRE 80-25	84	41.4a±3.91	28.8c±0.89	58.4b±10.0	18.5b±2.6	30 (22.9)
GR 30-63	95	36.9b±2.24	30.6ab±0.84	58.5b±0.33	22.4bc±6.6	61 (46.6)
GRE 80-2	95	36.7b±1.75	29.5bc±0.91	30.8c±5.36	22.9bc±1.3	28 (21.4)
GR 80-3	105	30.2c±1.25	31.9a±0.38	10.6d±1.10	6.2c±0.2	21 (15.9)

Note: Numbers in column with the same letter are not significantly different by DMRT =5%; * Fruit of mutant as result of artificial crossing with pollen of octoploid plants and each ploidy mutants produced about 2-36 fruit; % = percentage of seed number in mutant to wild type plant

exception for stomata. Decreasing stomata number per unit area in mutant plants was compensated with length of stomata and bigger size of aperture area (Table I). Increase in chromosome number affected the cell size. Rayburn *et al.* (2009) identified that stomata size was correlated with genome size of *Micanthus.* Vandenhout *et al.* (1995), Gao *et al.* (1996), De Oliveira *et al.* (2004), Rauf *et al.* (2006), Vanstechelman *et al.* (2009), Rayburn *et al.* (2009) and Laere *et al.* (2010) found similar results. Even though low density of stomata was compensated with bigger aperture area, the higher ploidy level had persistent slower growth. Warner and Edwards (1993) noted the photosynthetic rate per cell and the cell volume was doubled in polyploid than diploid, but decrease in cell number per unit leaf area was not proportional with increasing ploidy level. Eventually the photosynthetic rate per leaf area as the product of the rate per cell and number of photosynthetic cell per unit area was low (Warner & Edwards, 1993). Therefore, polyploid plant had lower biomass production (Laere *et al.,* 2010), fewer shoots (Laere *et al.,* 2010) and leaves (Vanstechelman *et al.,* 2009; Laere *et al.,* 2010) than their diploid progenitors.

Flower characteristics of the ploidy mutant and wild type were shown in Table II. Flower structure is important in relation to pollen distribution and reception, and on the hermaphroditic plant species flowers have evolved to suit particular pollination mechanism (Ainsworth, 2000). Strawberry flower of mutant and wild type included in perfect (sepal, petal, carpel & anther) and hermaproditic (carpel and anther) flower. In this experiment, the size of mutant flower was bigger than wild type. In this study Mishiba and Mii (2000) noticed larger number of petals as well in portulaca. Increase in flower size also was accompanied by bigger size of pollen (Table II), which is in accordance to Mishiba and Mii (2000), Mehetre *et al.* (2006) and Omidbaigi *et al.* (2010). Pollen size increased linearly in accordance of ploidy level in DNKW001, but there was also difference in size in the same ploidy level. Therefore, the pollen size could be used as tool for distinguishing normal and polyploid plant, as well as morphological structures of pollen seem to be useful for differentiating taxa (Gunes, 2011). Considerable variation in pollen size eventually reduces fertility (Mehetre *et al.,* 2006).

The staining of pollen/viability (Zaman, 2006; Tome *et al.,* 2007) and pollen germination (Susin & Alvarez, 1997; Adaniya & Shira, 2001; Wang *et al.,* 2003; Sharafi, 2010) have been used for determining fertility of pollen. Zaman (2006) distinguished stainable pollen, consequent of starchy pollen, as indicator of viable pollen and germinating pollen as indicator of pollen fertility. The pollen was considered being germinated when pollen tube clearly emerge or size of pollen twice of normal one. The data showed that pollen viability and germination (in sucrose & Boron solution) of mutants had similar trend in leveling up of ploidy level, in which ploidy mutant was lower than wild type plants (Table II). Ottavio *et al.* (1992) noted a linear relation between pollen viability and germination capability in many fruit species and others plants. Low viability and germination of pollen in control was allegedly caused by high temperature in the Green house at the day time, as mentioned by Sharafi (2010) that germination capability of pollen depended on various factors, namely environmental factors, nutrition conditions and genotype as well. Percentage of pollen

Fig. 1: Stomata (A and B) and plastid (C and D) of octoploid (left) and ploidy mutant (right) of strawberry plants

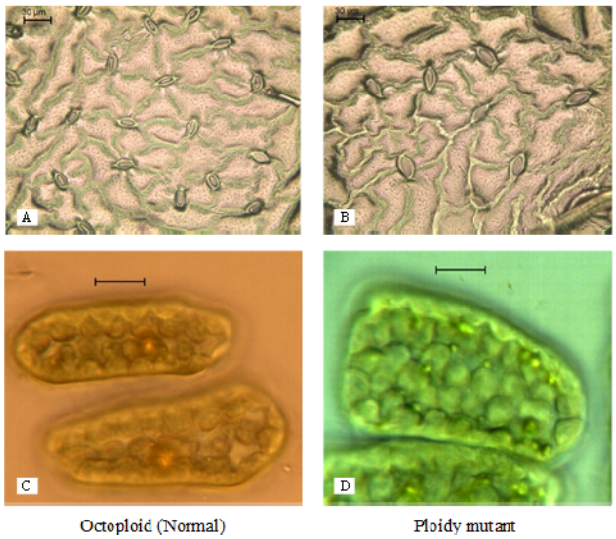
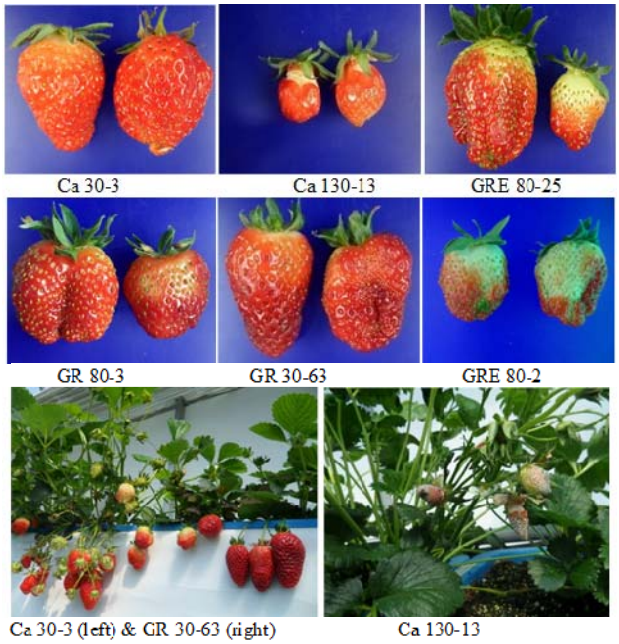


Fig. 2: Fruit of mutant plants as artificial pollination result with octoploid pollen



viability of DNKW001 in wild type and mutant was higher than Akihime and conversely was higher in wild type of Akihime for germination. Pollen viability and germination decreased linearly in mutant of DNKW001 with the exception of germination of GRE 80-25. Severe sterile, very low viability and no pollen tube grew out from pollen, occurred in Ca 130-13 and GR 80-3 in Akihime and DNKW001, respectively. Consequently, pollens were predicted absurd in fertilizing ovule. Lower pollen fertility in higher level ploidy plant had been also identified in portulaca (Mishiba & Mii, 2000) and melon (Susin & Alvarez, 1997). Meiotic aberration such as laggards, chromosome bridges, microculei, abnormal cytokinesis, chromatin pulling, meiotic restitution (Rezaei *et al.*, 2010), early chromosome migration in metaphases I and II and chromosome pairing anomalies (Tome *et al.*, 2006) are the factors reducing fertility. In this experiment it was found that the higher ploidy level of mutant (GR 30-63 & GRE 80-2) conferred higher germination percentage than lower ploidy of mutant (GRE 80-25). This exception was resembling in discovery tetraploid of ginger reporting that low fertility would determine the fruit set and seediness (Adaniya & Shirai, 2001).

Although pollen germination posed no problems (except Ca 130-13 & GR 80-3) in spite of in low percentage, natural crossing did not produce fruit and seeds. This is due to pollen dehiscence shortly after flowers open, while the stigmas receptive 2-3 days after flowers open even until wilted corolla (data not shown). According to Susin and Alvarez (1997), the pollen grain producing tube branching also decreases functionality. As a result, the ploidy mutant plant had significantly higher outcrossing rates (Husband *et al.*, 2008). In this experiment, artificial pollination as aforementioned was carried out. When the receptive flowers were pollinated with abundantly octoploid pollen, the fruit emerged with low density of seed and consequently the malformed fruits were produced (Fig. 2). Best form of fruit was shown by Ca 30-3 and GR 30-63 in Akihime mutant and DNKW001 mutants, respectively. The extremely bad condition with measly seed belonged to Ca 130-13 (Table II). The other mutants produced many seed but much lower than wild type, although the abundance of pollen was used in artificial pollination.

The ability of mutant to produce seed is a potency to use mutant in crossing with low ploidy level, and it could speed up the evolution in strawberry. Further exploration of advantages of higher ploidy level is needed to exploit the superiority for instance drought resistant like polyploid of *Coccinia palmata* and *Lagenaria sphaerica* (Ntuli & Zobolo, 2008) and *Capsicum annum* (Kulkarni & Borse, 2010). Unreduced gametes are relatively common in Fragaria, and allopolyploid and autopolyploid species have been discovered (Hancock, 1999). Moreover, hybridizations of diploid and higher-ploidy species have been succeeded (Bringhurst & Senanayake, 1966; Lei *et al.*, 2005). Spiegler *et al.* (1986) has also done crossing between *Fragaria x ananassa* (2n = 8x = 56) and *Fragaria vesca* (2n = 2x = 14) which produced decaploids after chromosome doubling (*Fragaria vescana* 2n = 10x = 70), and Yanagi *et al.* (2010) with artificial diploid×octoploid crosses. Therefore, variation in ploidy mutants as new strawberry genetic resources is essential for breeding activities (Ulukan, 2011), excepted Ca 130-13, which can also be involved in crossing with lower ploidy or non-cultivated strawberry in intercrossing specific traits or in constructing synthetic polyploid as asserted by Sangiacomo and Sullivan (1994).

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