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Study on chrysanthemum breeding by gamma (Co^{60}) irradiation on callus of 4 exotic varieties

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Four chrysanthemum varieties CN93, CN01, CN98 and CN20 were selected by Agricultural Genetics Institute (AGI), put into production which have been grown popularity in our country. However, they were cultivated for a long time, therefore these four varieties have already showed many limitations in the production and consumption process. In order to improve and overcome these limitations as well as change morphology of original varieties in accordance with the requirements of the current market, AGI used gamma irradiation (Co^{60}) on callus of 4 chrysanthemum varieties with radiation doses were 0 (original varieties), 5, 10, 20 and 30 Gy. To evaluate the initial results, we obtained 110 beneficial mutation lines with the morphologies such as: lower stem, shortener growth process, smaller leaf angle, changing the flower color, larger blooms and larger stem diameter than four original varieties. After observing and evaluating 110 mutation lines in comparison with original varieties by standard indicators of biological agriculture in the field, we selected 13 promising lines for further study.

Keywords: Chrysanthemum, mutation line, gamma irradiation, selection.

Introduction

Chrysanthemum is a kind of economic efficiency cut flower. Therefore, the breeders play an important role in new chrysanthemum varieties selection to meet the needs of customers. In recent years, mutagenesis method has brought many great achievements. By this method, Mandal *et al*. (2000) used gamma irradiation (Co^{60}) on callus of Morifolium chrysanthemum variety with different radiation doses and obtained the variation in leaf and flower colors. Datta, S.K, D. Chakrabarty and A.K.A Mandal (2001) irradiated gamma on callus and obtained some variation in flower color and flower structure (Datta, S. K. *et al*., 2001; Datta, S. K. *et al.*, 2005; Mandal *et al.* 2000)0. In Vietnam, Dao Thanh Bang *et al* selected and breeded the VCM1, VCM2 and VCM3 chrysanthemum by mutagenesis method on exotic chrysanthemum varieties (Bang D. T. *et al*. 2007)

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Four chrysanthemum varieties CN93, CN01, CN98 and CN20 were selected by Agricultural Genetics Institute (AGI) and recognized as an official variety by MARD, are now being put into production and have been grown popularity in our country. However, they were cultivated during a long time, therefore, these four varieties have already showed many limitations in the production such as: small stem and inflorescence (CN93), small leaf, a lot of axillary buds, small stem, long time variety, decentralized flower bloom (CN98), small number of petal, petal loose arrangement, asymmetric inflorescences (CN01), collapse stem, small petal in comparion with flower dimater (CN20).

Objectives: maintain the beneficial characteristics, overcome the variety limitations, as well as select the significant morphological traits meet the production requirements, we carried out to breed and select 4 chrysanthemum varieties by gamma (Co^{60}) irradiation on callus. After observing and evaluating in vitro and in vivo experiments, we selected promising lines for variety breeding and selection.

Materials and methods

The study involved 110 prospect mutation lines containing beneficial variations were obtained at M1V5 generation of 4 chrysanthemum varieties: CN93 (25 lines), CN01 (15 lines), CN98 (32 lines), CN20 (38 lines).

The experiment was designed sequentially, not repeat with the number of 500 plants/ lines. Standard indicators of biological agriculture were observed and assessed according to QCVN 01-58/2011/BNNPTNT test guidelines (MARD, 2011).

The mutant and control lines were grown with distance 15 x 15cm and oberverved sequencely 10 days per time. The care condition of mutant line and control is the same.

All chrysanthemums were lighted in the first 30 days, from 18 - 24h per day.

The observed indicators were evaluated according to the methodology of the International Centre for the flower trade flower production (ITC) in 2001 in Switzerland. The morphological characteristics and the number of mutations were observed and measured directly.

The observed indicators

+ The average of stem height (cm) = Total of stem height/ total of observed plant

+ The average of stem diameter (cm) = Total of stem diameter/ total of observed plant

+ Number of leaf = Total of leave number/ total of observed plant
+ Growth duration
+ Flower quality: Bloom diameter, flower color.
+ Flower long life (day)

The study was carried out from 8/2013 to 11/2014 at Flower and ornamental tissue culture room and Van Giang flower breeding farm - Department of Mutation and heterosis breeding - Agricultural genetics institute (AGI).

Data Analysis/ Statistical analysis

Analysis of variance was calculated for all data and comparisons between treatments were made at probability level $p \leq 0.05$ using Turkey's test and Microsoft excel.

Results

In order to assess and determine the genetic of significant morphology mutations such as: stem height, leaf number, stem and leaf characteristic, flower quality and growth duration of 4 chrysanthemum varieties in the field, we observed these growth and development characteristics of in vitro plant on M1V8 generation in the field.

Access the morphology, growth and development characteristics of CN93 beneficial mutation lines on M1V8 generation in the field

Table I: Standard indicators about growth of CN93 beneficial mutation lines on V8M1 generation

Mutant line	Stem height (cm)	Stem diameter (cm)	Leaf number (leaf)	Inflorescence/ bloom diameter (cm)	Flower long life (day)	Growth duration (day)
Control CN93	66,0±1,3	0,40±0,01	38,4±1,1	8,5±0,2	16,6±1,1	92,7±0,8
C5.3	64,2±1,6	0,55±0,02	36,9±1,2	9,3±0,3	16,2±0,8	92,7±1,1
C6.5	66,7±1,9	0,54±0,01	37,9±1,2	9,5±0,5	16,6±0,8	92,7±0,9

Fig. 1. Larger stem diameter (C5.3 mutant line)

The assessment results of 25 CN93 mutant lines showed: 3 lines (C7.1, C7.2 and C8) at 20 Gy dose were not maintained morphological characteristics from M1V5 generation. 22 other mutant lines were still contained significant morphology characteristics and showed following growth and development:

3 mutant lines (C1.1 – C1.3) at 20 Gy dose showed number of stem more than control (average of 2 – 4 stem/ plant). However, this flower quality was not high. Therefore, this can be indicated that mutant characteristics in this generation were inheriated from the previous generation. However the growth ability and flower quality were not sastify for breeding and selecting requirements, so these lines were kept such as new materials for plant breeding in the future.

11 mutant lines (C2.1 and C2.2 line at 10 Gy dose; C3.2 – C3.4 line at 20 Gy dose; C4.1 – C4.5 line at 30 Gy dose) showed short growth duration. These lines contained characteristics satisfied with the flower bed and potted flower orential. However, after observing growth standards, the results showed low flower quality, asymmetric inflorescences, decentralized flower bloom and short flower long life that affected flower economic value as well as not sastified for breeding requirements, so these lines were kept and observed furthermore. 8 mutant lines (C5.1 – C5.3 line at 20 Gy dose; C6.1 – C6.5 line at 30 Gy dose in this generation still maintained larger stem diameter than control (average of 0,48 – 0,55 cm, more than 0,08 – 0,15 cm in comparison with control). However, C5.3 and C6.5 line grew well such as: stem height and leaf number. These 2 lines showed normal growth duration (92,7 days). Besides, the results also showed centralized flower bloom and flower long life (16,2 – 16,6 days). In specially, the stem diameter of 2 lines more than control was 0,8 – 1,02 cm, petal closed arrangement. This is an important standard that affected

cut flower economic value. This can be indicated that these 2 mutant lines overcome partly variety limitation in the first step. So we can conclude that these 2 lines (C5.3 and C6.5 line) were promising lines for flower breeding. Other lines were not sastify for breeding and selecting requirements, so these lines were kept for plant breeding in the future.

Access the morphology, growth and development characteristics of CN01 beneficial mutation lines on M1V8 generation in the field

Table II: Standard indicators about growth of CN01 beneficial mutation lines on V8M1 generation

Mutant line	Stem height (cm)	Stem diameter (cm)	Leaf angle (^{o})	Leaf number (leaf)	Flower long life (day)	Growth duration (day)
Đ/C	67,3±1,6	0,50±0,01	60 - 70^o±3,2	37,2±1,5	16,6±1,1	93,8±0,9
C11.1	65,3±1,4	0,51±0,01	50 - 65^o±3,4	34,4±1,1	16,2±0,8	91,4±0,8
C11.2	66,1±1,3	0,51±0,01	53 - 65^o±3,7	35,9±1,6	16,2±0,8	90,8±1,0
C12.2	34,7±1,5	0,48±0,02	60 - 70^o±3,7	24,7±1,4	16,6±1,1	68,3±0,9
C13.1	35,0±1,4	0,50±0,02	60 - 70^o±3,3	25,3±0,9	16,6±1,1	65,7±0,9
C14.3	35,1±1,7	0,49±0,01	60 - 70^o±3,3	25,6±0,9	16,6±0,8	66,9±1,1

Fig. 2. Change the inflorescence colour (C13.1), smaller leaf angle (C11.1 line) and shorter stem (C12.2 line)

The results in table II showed that 14 mutant lines from CN01 were still maintained significant morphology characteristics from M1V5 generation and contained following growth and development:

5 mutant lines (C9.1 and C9.2 at 10 Gy dose; C10.1 - C10.3 lines at 30 Gy dose showed the larger stem diameter than control (average of 9,2 – 9,5 cm). However, after assessment of flower quality, the results indicated that this flower quality was not sastify. Therefore, we can conclude that these lines should be maintained and kept such as new materials for plant breeding in the future.

2 mutant lines (C11.1 and C11.2 line at 30 Gy dose showed leaf angle is smaller than control. This is an important characteristic because this can increase the plant density in an acreage unit and unaffected the photosynthesis ability. This made the real yield also. Besides, growth and development standard were stable in comparison with control (normal height (average of 65,3 cm and 34,4 leaves/ plant; centralized flower bloom; 8,1 – 8,9 cm bloom diameter; flower long life (16,2 days). This can be indicated that these 2 mutant lines were promising lines for new flower breeding.

7 mutant lines (C12.1 and C12.2 at 5 Gy dose; C13.1 and C10.2 lines at 20 Gy dose and 14.1 and C14.3 at 30 Gy dose) showed the stem height was shorter (30,8 - 35,1 cm) than control (32,2 - 36,5cm). Thís is firsly sastify the flower bed and potted flower requirement. However, after observing flower quality standards, we saw low flower quality, so these lines were not used as promising lines. 3 other mutant lines (C12.2, C13.1 and C14) grew and developed well. Besides, flower quality firsly sastified flower beds and potted flower standards such as: symmetric inflorescences, centralized flower bloom, bright color and long flower long life (16,6 days). So we assessed that 3 aboved lines were promising lines for plant breeding.

Access the morphology, growth and development characteristics of CN98 beneficial mutation lines on M1V8 generation in the field

Table III: Standard indicators about growth of CN98 beneficial mutation lines on V8M1 generation

Mutant line	Stem height (cm)	Leaf number (leaf)	Petal number/ flower (petal)	Stem diameter (cm)	Inflorescence diameter (cm)	Flower long life (day)	Growth duration (day)
Control CN98	73,4±1,5	41,4±1,0	292,0±10,6	0,60±0,01	11,6±0,2	16,0±1,1	105,6±1,0
C15.1	66,0±1,6	42,0±1,2	285,0±12,3	0,58±0,01	11,0±0,5	16,0±1,1	92,2±0,6
C16.9	68,2±1,8	41,4±1,5	289,0±12,7	0,62±0,01	12,2±0,5	16,0±0,8	92,0±0,6
C17.5	66,7±1,5	42,2±1,2	278,0±13,5	0,60±0,01	11,9±0,5	13,5±0,8	96,4±0,6

The results in table III showed that 32 mutant lines from CN98 contained following growth and development:

2 mutant lines (C15.2 and C17.4 at 20 Gy) dose were not maintained morphological characteristics from M1V5 generation. These characteristics were similar to control. 30 other lines still contained significant morphology

characteristics and showed following growth and development from M1V5 generation:

CN98 variety characteristics were long growth duration (100 – 110 days), good growth and development. However, this variety showed centralized flower bloom. This took long time for harvesting. This was also limitations that should be overcome variety. In the M1V8 generation, the growth duration of C15.3 - C15.8 lines and C16.1 - C16.8 lines decreased (89 - 95 days) but growth standard and flower quality showed limitations in comparison with the control. This can be concluded that: 14 these mutant lines had shortener growth duration but flower quality contained some limitations. Therefore, these lines should be maintained and kept for plant breeding. The growth duration of C15.1 and C16.9 lines were shorter than the control. This made flower stem height shorter as well (66 – 69 cm). This was not also affected flower quality and avoided collapsing in the production process. Stem diameter (0,58 – 0,62 cm) and bloom diameter (11 – 12,2 cm) were stable and similar to control. Besides, flower quality was high (bright color), flower bloomed centralizedly because of short growth duration. These standards played an important role in production. So we assessed that 2 aboved lines were promising lines for plant breeding.

The flower color of 14 mutant lines (C17.1 - C17.3, C17.5 - C17.7 at 20 Gy dose and C18.1 - C18.8 lines at 30 Gy dose) were changed. This was similar to results in the M1V5 generation. However, the growth ability of C17.1 - C17.3; C17.6; C17.7 and C18.1 - C18.8 lines was low, color of flower showed strong variation, flower bloomed incentralizedly. This can be concluded that these lines were not sastify breeding standards and should be kept and observed furthermore. C17.5 was promising line for breeding. The flower color of this line was changed in accordance with new plant breeding and the ability of growth was high, flower bloomed remarkedly centralizedly (stem height: 64 - 68 cm; growth duration: 95 – 98 days, long life of cut flower: 12 -15 days, bloom diameter: 11,5 – 12,3 cm).

Fig. 3. Larger inflorescence diameter (C16.9 line)

Access the morphology, growth and development characteristics of CN20 beneficial mutation lines on M1V8 generation in the field

Table IV: Standard indicators about growth of CN20 beneficial mutation lines on V8M1 generation

Mutant line	Stem height (cm)	Leaf number (leaf)	Flower number (Flower)	Stem diameter (cm)	Inflorescence diameter (cm)	Flower long life (day)	Growth duration (day)
Control	61,8±1,4	39,4±1,3	21,0±0,8	0,52±0,01	3,8±0,3	16,4±0,8	90,8±0,9
C22	61,2±1,3	35,8±1,1	21,7±0,8	0,48±0,02	3,7±0,2	15,2±0,8	90,2±0,5
C23.1	38,3±1,5	26,2±1,0	25,0±0,7	0,50±0,01	3,6±0,3	19,0±0,8	69,4±0,9
C25.1	62,0±1,5	39,0±1,1	26,5±1,1	0,60±0,02	3,8±0,4	15,8±0,8	91,4±0,9

The assessment results of 39 CN20 mutant lines in the M1V8 generation showed: 3 lines (C19.1, C20.2 and C21.2) were not maintained morphological characteristics from M1V5 generation. 36 other mutant lines were still contained significant morphology characteristics from M1V5.

The flower color of C22 line (at 20 Gy dose) was changed (slight color) in comparison with the control (white). Besides, growth and development characteristics showed stablely and strongly (stem height: 61,2 cm; leaf number: 35,8 leaves/ plant, bloom diameter: 4,2 – 5,0 cm; flower long life: 15,2 days). This can be concluded that this is a promising line and should be observed the color in the next generation.

The stem height of 13 lines (C23.1 - C23.13 at 30 Gy dose) was shorter than control. However, only C23.1 line contained characteristics in accordance with potted flower and flower bed standards such as: dark geen leaf, the stem can be divided in to many branches, flower number/ plant was 21 -23 flowers; flower long life was 18 – 20 days, plant shape was extremely symmatic. These were accordance with potted flower and flower bed standards. The other lines contained characteristics were not accordance with breeding requirements and kept and observed furthermore.

The flower/ plant number of 22 lines was more than the control included: 12 lines (C24.1 - C24.12 lines at 20 Gy dose); 10 lines (C25.1 - C25.10 at 30 Gy dose). Other lines (C24.1 - C24.12, C25.2 - C25.10) contained instable growth standard. So these lines need to be kept for breeding in the future. The flower/ plant number of C25.1 was 25 – 27, the stem diameter of this line was stable and more than control (0,1 – 0,13 cm). This made flower stem harder and

stronger. In specially, growth duration was shortened (90 – 92 days) and flower bloomed centralizedly. This can be indicated that this is promising mutant line overcome variety limitation in the first step.

Conclusions

All mutant lines were grown and developed well at in vitro period in the nursery.

13 prospect mutant lines were selected from 110 significant morphological lines on M5V5 generation:

CN93 (2 lines): C5.3 (20 Gy) and C6.5 (30 Gy) line: larger stem and inflorescene diameter.

CN01 (5 lines): C11.1 and C11.2 (30 Gy) line: smaller leaf angle, C12.2 (0,5 Gy), C13.1 (20 Gy), C14.3 (30 Gy): shortener stem.

CN98 (3 lines): C15.1 (20 Gy) and C16.9 (30 Gy) lines: shortener growth duration (90 - 94 days), C17.5 (20 Gy): darker yellow inflorescene.

CN20 (3 lines): C22 (20 Gy): change colour of inflorescene into yellow, C23.1 (30 Gy): shorter stem, C25.1 (30 Gy): increase the number of flower, larger stem diameter

Carry on next studies: evaluate and select on M1V9 generation, observe the prospects characteristics and select beneficial mutation lines for production as well

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