Indian J. Hort. 72(1), March 2015: 77-83

Induction of genetic variability through gamma irradiation in mini marguerite (*Chrysanthemum paludosum* Poir.) and their RAPD-based genetic relationship

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ABSTRACT

The present investigation was conducted during the *kharif* season of 2010-2012 to study hormesis, morphological and biochemical attributes associated with mutation and purification of novel types in mini marguerite. The seeds of *Chrysanthemum paludosum* were exposed to gamma rays (source ^{60}Co) at 20, 40, 60, 80 and 100 Gy dose treatments and sown along with control (un-irradiated seeds). Lower doses of irradiation resulted in hormesis and induced encouraging novelties, while the higher doses (100 Gy) induced elevated degree of abnormalities (6.41%), which consequently led to mortality (52.50%). Morphological characters, *viz*., plant height, plant spread (E-W and N-S), delayed flowering was observed in all the treatments. Biochemical characters, *viz*., chlorophyll *a* and *b* content as well as total chlorophyll content increased. The per cent abnormal plants, maximum deformed, per cent abnormal leaves, flower head fasciation/ asymmetrical flower heads was also observed all the treatments. Two promising mutants, *viz*., Star type (P_1) at 40 Gy and Jasmine type (P_2) at 60 Gy gamma irradiation treatment were tagged, screened and checked for stability of characters for genetic study and possible uses of the traits. The seeds of M_2 and M_3 generations were sown to observe their morphological characters and mutants in each population. RAPD molecular marker was used to study the genetic divergence and establishment of distinctiveness between the mutants developed, as a result of treatment of gamma rays. The results will aid in development of efficient germplasm utilization and management strategies.

Key words: *Chrysanthemum paludosum*, gamma rays, irradiation, mutants, RAPD.

INTRODUCTION

Chrysanthemum paludosum Poir. [*Leucanthemum paludosum* (Poir.)] commonly called Mini marguerite, Baby Marguerite, White Buttons and Snow Daisy belongs to family Asteraceae is native to Western Europe. It is a vigorously growing, hardy herbaceous annual. In floriculture industry, there is always a craze for developing novelties to replace older varieties with newer ones. Since in ornamentals, a variety cannot maintain interest for a long time, and people have the desire for newer forms through various methods of breeding. The possibilities of mutation breeding are favourable for various reasons such as the usually large heterozygosity of the material which allows direct detection of mutations in the irradiated material, with the intention of improvement in visible characteristics (Broertjes, 3).

Scientific interest in breeding using mutagens has drastically decreased during the last two decades. Interest in research has shifted towards development and application of molecular techniques as tools in breeding and genetic transformation of plants, since they allow more directed approach in pursuance of breeding goals. Molecular techniques generate high developmental costs and require sophisticated equipments and a highly trained staff, also the investment in such expensive methods does not seem adequate in case of ornamental crops with their limited economic importance as compared to agricultural crops (Schum, 14).

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MATERIALS AND METHODS

The present investigation was carried out at the Model Floriculture Center, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during 2010-12. The experimental material comprised of the seeds of *Chrysanthemum paludosum* variety Snow White. The seeds of the parental line were procured from Horti Flora Seed Farms, Patiala (Punjab) and were exposed to gamma rays (^{60}Co) at 20, 40, 60, 80 and 100 Gy dose treatments at gamma irradiator facility of National Botanical Research Institute, Lucknow. The gamma irradiated seeds (M_1) along with the control (un-irradiated seeds) were sown on raised nursery beds and transplanted in experimental field in randomized block design with three replications. The plot size was 1000 cm ×

1800 cm with 1200 seedlings/plot with plant spacing of 50 cm × 30 cm. All the recommended package of practices were followed throughout the growing period. Morphological and biochemical observations were recorded for 13 traits from randomly selected three plants per treatment per replication. The chlorophyll content of the leaves was estimated as suggested by Hiscox and Israelstam (8). Visual observations on different characters were made and the plants showing change in form of flowers, florets, etc. were critically observed and any abnormality observed in the plants of M_1 generation in different treatments was tagged, screened and recorded. At maturity, each mutant plant was individually harvested and the seeds were tagged subsequently for sowing the next generation.

The M_2 seeds along with the control (un-irradiated seeds) were sown on raised nursery beds and transplanted in experimental field in randomized block design with three replications. The plot size was 100 cm × 180 cm with 12 plants/plot with spacing of 50 cm × 30 cm. All the recommended package of practices were followed throughout the growing period. M_2 mutants were periodically observed right after germination and were tagged for subsequent observations. The data was recorded for randomly selected three plants per treatment per replication and average was computed. Any abnormality or variation observed in the plants of M_2 generation was screened and tagged for subsequent observations. At maturity, each mutant plant was individually harvested and the seeds were tagged and screened subsequently for sowing the next generation (M_3) and checked for the stability of the characters. The data generated were subjected to the statistical analysis in accordance with the procedure outlined by Gomez and Gomez (6).

The genomic DNA was extracted by using the CTAB method (Doyle and Doyle, 4) with slight modifications. PCR amplification was performed (William *et al.*, 15) with arbitrary decamer primers. Band sharing data was analyzed to obtain genetic similarities based on Jaccard's similarity coefficient (Jaccard, 9) among the isolates by using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc, version 2.2) (Rohlf, 13). UPGMA (Unweighted Pair Group Method using Arithmetical Averages) algorithm was employed to determine the genetic relationship of the parent and the mutant genotypes in *C. paludosum*.

RESULTS AND DISCUSSION

The observations made on several un-irradiated plants of *C. paludosum,* raised in the experimental field presented in Table 1; indicate the different parameters pertaining to morphology. The normal plants of *C. paludosum* grew to a mean plant height

Table 1. Effect of gamma irradiation on different characters of *Chrysanthemum paludosum*.

Character	Control	Gamma irradiation (Gy)					CD (5%)	CV (%)
		20	40	60	80	100		
Plant survival (%)	100 (90)*	81.17 (64.50)*	71.67 (57.85)*	66.67 (54.80)*	58.17 (49.71)*	47.50 (43.56)*	3.50	2.71
Plant abnormality (%)	0.00 (0.00)*	1.90 (7.77)*	2.24 (8.55)*	4.08 (11.63)*	4.61 (12.30)*	6.41 (14.66)*	1.57	26.9
Plant height (cm)	47.83	45.17	42.5	41.17	39.6	38.83	5.11	6.6
Plant spread (E-W) (cm)	66.33	64.13	63.33	61.8	57.27	55.43	4.57	4.09
Plant spread (N-S) (cm)	51.00	48.83	46.43	45.33	44.1	43.3	7.13	8.43
Leaf length (cm)	4.27	4.17	4.09	3.86	3.69	3.33	0.53	7.43
Leaf width (cm)	2.23	2.17	2.13	1.9	1.86	1.81	NS	11.4
Days to flowering	118.9	120.4	121.87	124.87	127.9	131.4	2.33	1.03
Chlorophyll *a* (mg/g)	2.469	2.54	2.65	2.67	2.668	2.717	0.04	0.8
Chlorophyll *b* (mg/g)	0.652	0.649	0.682	0.703	0.714	0.714	0.02	1.93
Total chlorophyll (mg/g)	3.102	3.17	3.311	3.352	3.36	3.41	0.05	0.85
Abnormal leaf (%)	0.00 (0.00)*	1.07 (5.87)*	2.51 (9.11)*	5.07 (12.88)*	6.09 (14.27)*	7.39 (15.76)*	1.65	24.7
Abnormal flower (%)	0.00 (0.00)*	2.13 (8.31)*	4.50 (12.24)*	5.70 (13.80)*	6.50 (14.74)*	6.83 (15.13)*	1.33	17.1

*Angular transformed values

of 50.75 cm with plant spread of 66.33 cm (E-W) and 52.83 cm (N-S). The leaves were alternate, green, lack punctate glandular hairs, glabrous, non-succulent, dentate-serrated, smooth, pinnatified. The mean leaf length was 4.33 cm, leaf width 2.28 cm and leaf area of 7.29 cm^2. The size of radiate capitula (diameter) was 3.28 cm, weighing 1.17 g and disc was 1.62 cm across. Each plant had a large number of pedunculate, radiate capitula (72.33) with 22.83 ray florets and 242.17 disc florets. The weight of ray florets was 6.25 mg and that of disc florets was 1.01 mg. The ray florets were 1.17 cm long and 0.57 cm broad borne on the flower head which was 1.45 cm high. Cent per cent plant survival was recorded in control, which declined with increase in dose of gamma rays, with minimum (47.50%) when exposed to 100 Gy. Reduction in plant survival after exposure to gamma rays has been explained to be due to disturbances of auxin synthesis and chromosomal aberration (Gunckel and Sparrow, 7).

Significant reduction in plant height was observed with increase in the dose of gamma rays irradiation. The maximum plant height in control (47.83 cm) and the minimum in 100 Gy (38.83 cm) were recorded. Significant reduction in the plant spread (E-W and N-S) was observed in *C. paludosum* with the increase in the gamma irradiation dose. Significant delay in flowering over the control was observed, with earliest blooming in control, while the maximum days to bloom were recorded with 100 Gy treatment. The delay in bud initiation ultimately resulted in late blooming, which may be due to reduction in the rate of various physiological processes and inhibition of growth and the plant remained in juvenile stage and thus unable to differentiate flower heads due to gamma irradiation. Due to irradiation, many biosynthetic pathways are altered which are directly and indirectly associated with the flowering physiology and plant morphology (Mahure *et al.*, 11). Significant reduction in size of the leaf, with increase in dose of gamma irradiation was observed. This may be due to the poor growth of plants due to radiation damage (Gaul, 5).

The chlorophyll content was influenced significantly by various gamma rays treatments. Increase in the chlorophyll content was observed with increase in the gamma irradiation dose. Datta (3) also observed similar results and reported that the basic cause of abnormalities is associated with physiological disturbances of growth substances, change in enzymes activities, variation in ascorbic acid concentration, breakage of phosphate metabolism, accumulation of free amino acids, etc.; incited by X-rays and colchicine. The per cent abnormal plants significantly increased with the increase in gamma rays treatment over the control. Among the different gamma rays treatments, maximum deformed plants were recorded with 100 Gy gamma rays treatment (Fig. 2) and none in control. The significant production of abnormalities may be due to radiation damage of the irradiated plants particularly chromosomal breakage (Gaul, 5), which causes physiological, morphological and cytological disturbances by gamma radiation. Per cent abnormal leaves significantly increased with the gamma rays treatment over the control. The different types of leaf abnormalities included change in leaf shape and size, margins, apex, fission and fusion were recorded after irradiation. There was no dose specific or variety specific abnormalities in leaves. Leaf variegation was observed in one plant of *C. paludosum* (Fig. 2a) in 40 Gy treatment. Significant increase was found in per cent plants with flower head fasciation /asymmetrical flower heads due to irradiation and again these were not dose specific. Flower heads became fasciated in different forms (Fig. 2b). These abnormalities were genotype dependent and mechanism may be involved in the repair of radiation induced damage within the organism (Banerji and Datta, 1).

Visual observations on different characters were made and the plants showing change in form of flowers, florets, etc. (Fig. 1 & 2), were critically observed and the type of form other than normal were tagged and recorded. The plants were also observed for any chimera which could be used for chimera management through vegetative propagation or through tissue culture techniques. The change in flower form was also recorded by Lamseejan *et al.* (10) in *C. morifolium*. Two mutants, *viz*., Star type (P_1) at 40 Gy and Jasmine type (P_2) at 60 Gy, which were true breeding were screened, tagged and checked for the stability of the characters. The observations were recorded on morphological characters of the mutants developed after gamma irradiation in M_2 and M_3 generations and the mean values of the randomly selected three plants per treatment per replication are presented in Table 2 and Fig. 1.

Mutant P_1 was developed at 40 Gy gamma rays irradiation treatment and differed in many characters over the original parent plants was dwarf with reduced plant spread (E-W and N-S), lesser leaf length, width and area than the original. The number of flowers per plant was also reduced with smaller Star shaped flower. The flower head weight, ray floret weight and disc floret weight were also reduced along with the length and width of the ray florets but the flower had slightly more flower head height than that of the parent genotype.

Mutant P_2 was developed in *C. paludosum* at 60 Gy gamma rays irradiation treatment hav low plant height, reduced plant spread (E-W and N-S) and

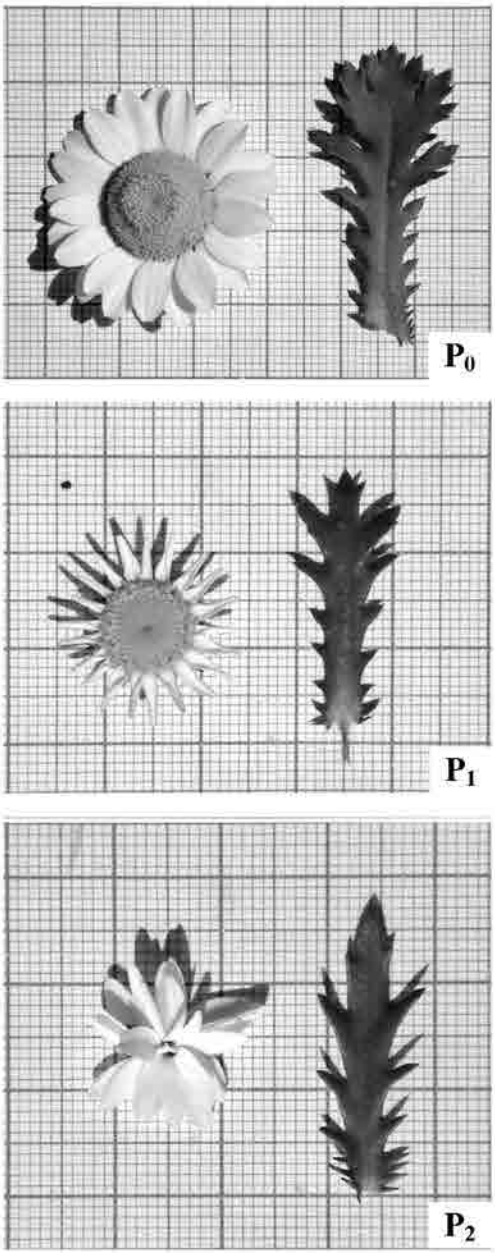

Fig. 1. Capitula and leaf of un-irradiated *Chysanthemum paludosum* (P_0) and its two mutants gamma irradiation induced mutants, *viz.*, P_1 (Star type) and P_2 (Jasmine type).

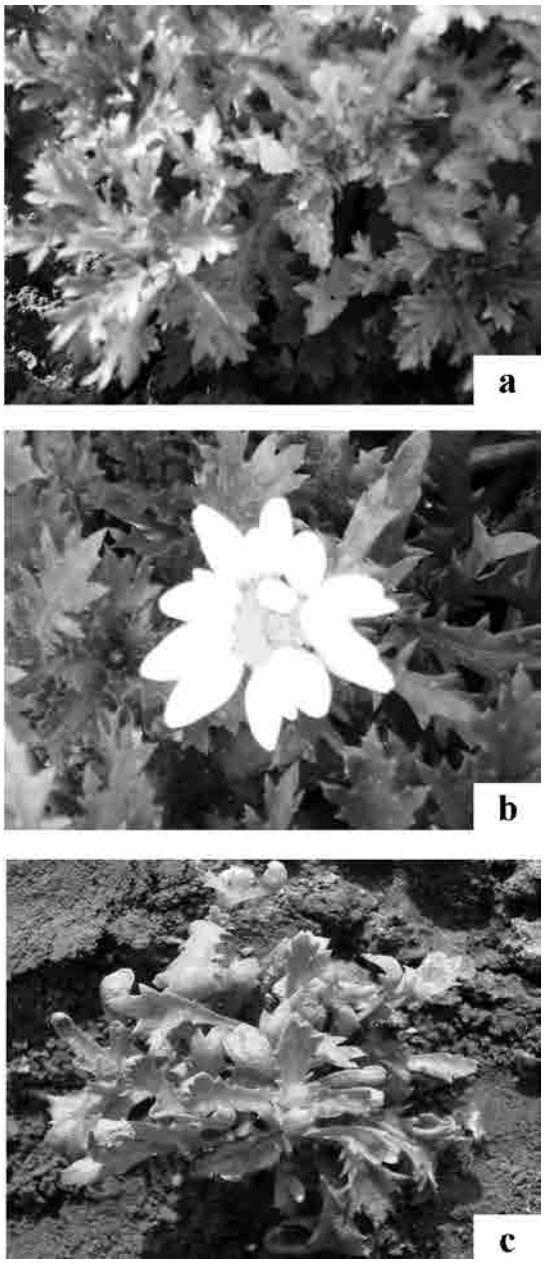

Fig. 2. Abnormalities due to gamma irradiated *Chysanthemum paludosum.* a = Variegated plant at 40 Gy; b = Flower head fasciation at 60 Gy; c = Deformed plant at 100 Gy.

smaller leaf length, width and area than the original. The number of flowers per plant was reduced with smaller jasmine type flowers, smaller flower head diameter, disc diameter, lesser number of ray and disc florets. The flower head weight, ray floret weight and disc floret weight were also lower along with lower length and width of the ray florets but the flower had more flower head height with changed flower form from single to double.

From the present analysis of original species and their gamma rays induced mutants, it has been found that in addition to change in flower shape, significant changes in some morphological characters had occurred in the mutants. This suggests that the changes were perhaps induced at several independent loci. However, pleiotropic or epistatic effects of a

Table 2. Morphological characters of original species and its mutants developed after gamma irradiation in *C. paludosum*.

Character	Parent genotype	Mutant	
		P_1 (Star type)	P_2 (Jasmine type)
		40 Gy	60 Gy
Plant height (cm)	50.75	41.50	38.00
Plant spread (E-W) (cm)	66.33	58.67	52.83
Plant spread (N-S) (cm)	52.83	44.50	41.83
Leaf length (cm)	4.33	2.72	2.70
Leaf width (cm)	2.28	1.30	1.01
Leaf area (cm^2)	7.29	2.88	2.18
No. of flowers/ plant	72.33	65.63	56.17
Flower diameter (cm)	3.28	1.76	1.68
Disc diameter (cm)	1.62	1.09	0.25
No. of ray florets	22.83	21.00	16.83
No. of disc florets	242.17	164.27	39.26
Head weight (g)	1.17	0.63	0.97
Ray floret weight (mg)	6.25	3.35	5.60
Disc floret weight (mg)	1.01	0.64	0.42
Ray floret length (cm)	1.17	0.63	0.92
Ray floret width (cm)	0.57	0.2	0.38
Head height (cm)	1.45	1.69	2.31
Flower form	Single	Single	Double

mutant gene controlling different characters cannot be ruled out. Gamma irradiation induced new flower shape mutants of the present experiment may find very useful in future practical breeding programmes and the new mutants can also be used directly for floriculture industry/trade.

PCR amplification of DNA extracted from the mutants and the original species of *C. paludosum* was performed with two random decamer primers (LC-94 and LC-86). The amplification profile generated by each primer was compared and the relative molecular size of each band was examined by comparing with DNA size marker. The number of RAPD loci scored and polymorphism detected by the two primers in the mutants and the parent species are presented in Table 3. The total numbers of 16 loci were amplified from two primers (Fig. 3). This gave an average of 5.5 loci per primer with an average polymorphic percentage of 64.58. Based on polymorphism percentage and unique band amplification, all the primers were considered highly informative primers with average PIC value of 0.39.

The dendrogram generated using SAHN cluster analysis and UPGMA method illustrated in Fig. 4 and the matrix of the Jaccard's similarity coefficient of the mutants of *C. paludosum* based RAPD markers (Table 4) revealed that the dendrogram separated the original species of *C. paludosum* and its two mutants into two major clusters A and B, at the demarcation of approximately 45% genetic similarity. Cluster A consisted of the original species, while the cluster B consisted of its two mutants (P_1 and P_2) with approximately 63% genetic similarity. The UPGMA dendrogram based on RAPD analysis indicated that the mutants are fairly distant from the parents and also among themselves. Knowledge of the genetic relationship between the two mutants, contributing to genetic diversity can greatly aid the development of efficient germplasm utilization and management strategies. The variability that is introduced by mutation breeding includes flower form variation which is quite divergent when compared to the parent. The percentile variations in the mutated population were studied to decipher the extent of variation the mutagen has brought about at the molecular level. DNA based markers are suggested as key strategy to determine the cultivar purity leading to improve IPR mechanism (Riek, 12).

Table 4. Matrix of Jaccard's similarity coefficient of *C. paludosum* and its mutants based on RAPD markers.

	1	2	3
1	1		
2	0.474	1	
3	0.421	0.632	1

1 - *C. paludosum*, 2 to 3 - Mutants of *C. paludosum* P_1 to P_2

Table 3. Details of RAPD primers used for the molecular characterization of mutants of *Chrysanthemum paludosum.*

Code	Sequence (5′ to 3′)	%GC	MMB	PMB	% Poly	PIC	H_i	Rp	D	D_L
LC-94	$^{5'}$GTCGCCGTCA$^{3'}$	70	3	6	66.67	0.44	0.20	4	0.63	0.28
LC-86	$^{5'}$GTTGCGATCC$^{3'}$	60	3	5	62.50	0.33	0.23	3.3	0.40	0.20
	Average		3	5.5	64.58	0.39	0.22	3.7	0.46	0.24

MMB = Monomorphic bands; PMB = Polymorphic bands; %Poly = % Polymorphism; PIC = Polymorphic Information Content; H_i = Average expected gene diversity; Rp = Resolving power; D = Discrimination power; D_L = Discriminating power

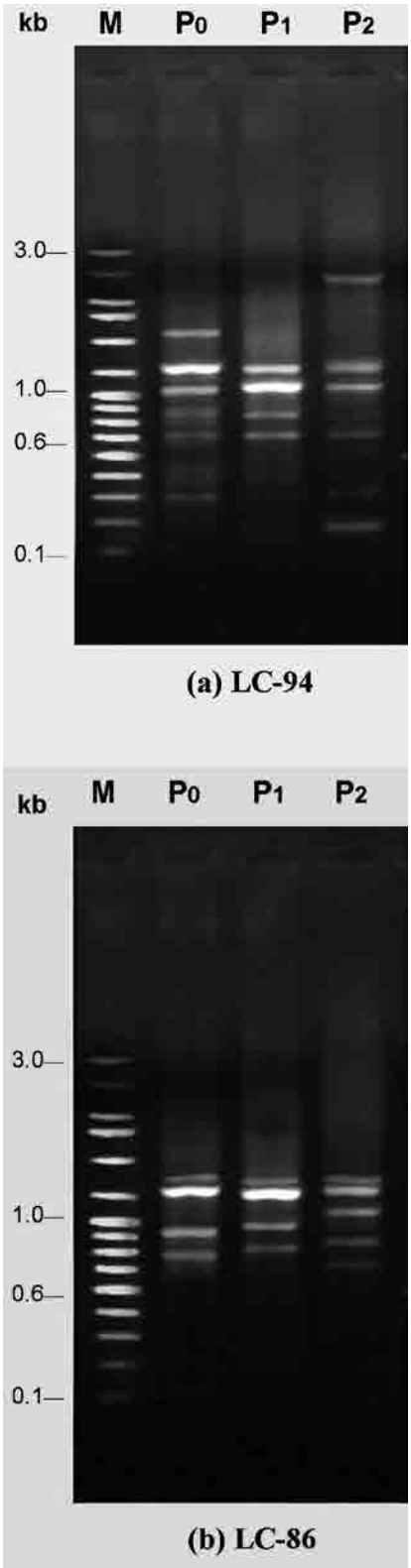

Fig. 3. Molecular diversity generated among *Chysanthemum paludosum* and its two mutants by RAPD primer (a) LC-94 and (b) LC- 86.

Our present study clearly indicates that RAPD markers can be used for genetic diversity studies among the radiation induced mutants and the parent species at genomic level, suggesting that by using RAPD molecular markers, the newly evolved mutants can be easily differentiated from their parents. This would be a very useful tool in identifying and protecting them from possible infringements and for protecting the Plant Breeders' Rights.

ACKNOWLEDGEMENT

The corresponding author is grateful to University Grants Commission, New Delhi for awarding Teacher Fellowship under Faculty Improvement Programme.

REFERENCES

1. Banerji, B.K. and Datta, S.K. 2001. Induction and analysis of somatic mutation in *Chrysanthemum* cv. 'Surekha'. *J. Nucl. Agric. Biol*. **30**: 83-90.

2. Broertjes, C. 1968. Mutation breeding of vegetatively propagated crops. **In**: *Mutation in Plant Breeding*. II Proc. FAO/IAEA, Milan, Italy, pp. 139-65.

3. Datta, S.K. 1988. 'Agnishikha' - A new chrysanthemum cultivar evolved by gamma irradiation. *Floriculture*, **9**: 10.

4. Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissues. *Focus*, **12**: 13-15.

5. Gaul, H. 1970. Plant injury and lethality. **In**: *Manual on Mutation Breeding*, Vienna, FAO/ IAEA, pp. 85-90.

6. Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research* (2nd Edn.), John Wiley and Sons, New York.

7. Gunckel, J.E. and Sparrow, A.H. 1961. Ionizing radiations: Biochemical, physiological aspects of their effects on plants. *Encyl. Plant Physiol*. **16**: 555-617.

8. Hiscox, J.D. and Israelstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian J. Bot*. **57**: 1332-34.

9. Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaudoise Sci. Nat*. **44**: 223.

10. Lamseejan, S., Jompuk, P. and Deeseepan, S. 2003. Improvement of chrysanthemum variety 'Taihei' through *in vitro* induced mutation with

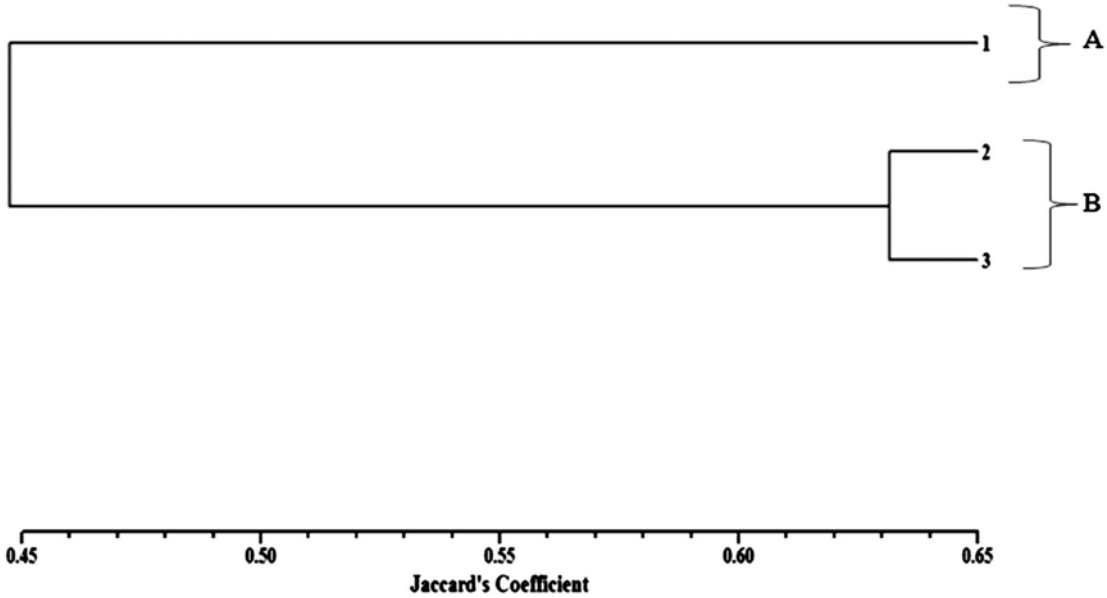

Fig. 4. Dendrogram depicting the classification of *Chysanthemum paludosum* and its two mutants based on RAPD. 1 - *C. paludosum*, 2 to 3 - Mutants of *C. paludosum* P_1 to P_2

chronic and acute gamma rays. *J. Nuclear Soc*. (*Thailand*), **4**: 2-13.

11. Mahure, H.R., Choudhry, M.L., Prasad, K.V. and Singh, S.K. 2010. Mutation in chrysanthemum through gamma irradiation. *Indian J. Hort*. **67**: 356-58.

12. Riek, D.J. 2001. Are molecular markers strengthening plant variety registration and protection? **In**: *Proc XX EUCARPIA Symposium Strategies for New Ornamentals*, Part I, Van Huylenbroeck, J., Van Bockstaele, E. and Debergh, P. (Eds.). *Acta Hort*. **552**: 215-23.

13. Rohlf, F.J. 2009. NTSYSpc: Numerical Taxonomy and Multivariate Analysis Systems. Version 2.2E. Exeter Publishing Ltd., Setauket, New York.

14. Schum, A. 2003. Mutation breeding in ornamentals: an efficient breeding method. *Acta Hort*. **612**: 47-60.

15. William, J.G., Kubelik, A.R., Livak, K.J. and Raafalski, J.A. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nuc. Acid Res*. **18**: 6531-35.

Received : December, 2013; Revised : August, 2014;
Accepted : November, 2014