"MUTATION BREEDING IN ROSE (*Rosa indica* L.)"

By

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DEPARTMENT OF HORTICULTURE

COLLEGE OF AGRICULTURE, PUNE.

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MAHARASHTRA STATE (INDIA)

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By

Mr.Bhagat Sangram Ramdas

B.Sc. (Agri)

In partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE (HORTICULTURE)

In

FLORICULTURE AND LANDSCAPING

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CANDIDATE'S DECLARATION

I hereby declare that, this thesis entitled, **"Mutation Breeding in Rose (*Rosa indica*L.)"**has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place: College of Agriculture, Pune

Date: / / 2015 **(BHAGAT S. R.)**

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CERTIFICATE

This is to certify that the thesis entitled**, "Mutation Breeding in Rose (*Rosa indica* L.)"**submitted to Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE (HORTICULTURE) in Floriculture And Landscaping,** embodies the results of a piece of bona-fide research work carried out by **Mr.BHAGAT SANGRAM RAMDAS**, under my guidance and supervision and that no part of the thesis has been submitted for any other Degree, Diploma or publication.

The assistance and help rendered during the course of this investigation have been duly acknowledged.

Place: Pune
Date: / / 2015

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CERTIFICATE

This is to certify that the thesis entitled,**"Mutation Breeding in Rose (*Rosa indica* L.)"** submitted to the Faculty of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE) in FLORICULTURE AND LANDSCAPING,** embodies the results of a piece of bona-fide research work carried out by **Mr. BHAGAT SANGRAM RAMDAS,** under the guidance and supervision of **Prof. S. K. CHAVAN,** Assistant Professor of Horticulture, College of Horticulture, Pune and that no part of the thesis has been submitted for any other Degree or Diploma.

Place : Pune
Date : / / 2015

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The present investigation on "Mutation Breeding in Rose (*Rosa indica* L.)" was under taken during 2013-14 at Department of Horticulture, College of Agriculture, Pune-411005. The experiment was laid out in completely randomized design (CRD) with nine treatments and four replications. Well developed buds were treated by dipping the scion wood with buds in various concentrations of EMS and MMS (0.20%, 0.25%, 0.30%, 0.35%) respectively for 8 hours. The treated and control eye buds were budded on the same

day on root stock *Rosa multiflora* and evaluated for various morphological characters.

The observations recorded on plant height, plant spread, leaf area, number of thorns on flower stalk, days required for first flower bud initiation, average weight of flower, length of peduncle, number of petals per flower, vase life and petal area were significantly influenced by chemical mutagens and their different concentrations. The results on days required for bud sprouting and colour of flower were not found significant.

Among the various concentrations of two chemical mutagens the results of treatment T_1 (EMS 0.20%) was found to be promising for plant spread, leaf area, average weight of flower, number of petals per flower, length of peduncle, vase life and petal area.

It can be concluded that treatment of EMS 0.20% was found to be best for growth and flowering in rose.

1. INTRODUCTION

All over the world, the floricultural sector is experiencing rapid changes. Due to globalization and its effect on income generation in different parts of the world the per capita consumption of flowers in most of the countries is increasing. Besides traditional centers of production (The Netherlands, Columbia. Israel and Kenya), new production centers are developing in Latin America, Africa and Asia. The floriculture production is increasing many times compared to a decade ago. The Asian countries like India, China, Korea and Vietnam, etc., are moving in the direction of more intensive floriculture.

Flowers are associated with mankind from the dawn of civilization. They form the soul of garden and convey message of nature to man. It is truly a symbol of affection, beauty, friendship and love. Thus it is inseparable part of human life.

Flowers have been associated with our social life since ancient time and are used for divine purpose. India has a rich heritage of ornamental horticulture. Flowers are symbol of beauty and love. In our country, flowers are commonly used in homes and temples. We are intimately associated with flowers and on all occasions in marriages, religious ceremonies and social functions.

Commercial flower cultivation in India is a recent development and taking a momentum. There is an increasing demand for Indian roses, carnations, chrysanthemums, orchids, gladiolus and exotic flowers in foreign countries

mostly western nations which give commerce ministry the sweet whiff of goldmine.

Rose has always been the most favorite flower in the world. Rose cut flowers play an important role in interior decoration and add charm to different occasions like marriage ceremonies, on arrival and departure of different dignitaries, gift on birthdays, valentine's day etc. Flowers are mentioned as the social fabric of our country.

Rose belongs to family *rosaceae.* Roses (*Rosa indica*) have a long and colorful history. Chromosome number of rose is 2n=14. It is a perennial plant and propagated by T-budding and shoot tip grafting. They have been symbols of love, beauty and war. Rose is referred as "Queen of flowers" as well as "King of flowers". Rose possess different characters required for cut flowers such as more number of petals, slow opening of bud, more longevity and attractive colours. Roses are hardy and can better withstand stress of unfavorable weather conditions.

Rose, perennial shrub or vine of the genus *Rosa,* within the family Rosaceae, an almost universally distributed group of 100 species. The great majority are native to Asia. Many are cultivated for their beautiful, fragrant flowers. These are commonly white, yellow, orange, pink, or red and, in wild roses, they are borne singly or in small clusters. The flowers of wild roses usually have five petals, while the flowers of cultivated roses are often double (i.e., with multiple sets of petals).

Roses are erect, climbing, or trailing shrubs whose stems are usually copiously armed with prickles of various shapes and sizes that are called thorns. The plant's leaves are alternate and pinnately compound (i.e., feather-formed). The rather oval leaflets are sharply toothed. The rose plant's have fleshy, sometimes edible, berrylike "fruit" (actually the floral cup) is known as a hip.

Roses are native primarily to the temperate regions of the Northern Hemisphere. Most rose species are native to Asia, with smaller numbers being native to North America and a few to Europe and Northwest Africa. Roses from different regions of the world hybridize readily, giving rise to types that overlap the parental forms, and making it difficult to determine basic species. Of the more than 100 species of roses, fewer than 10 species (most native to Asia) were involved in the crossbreeding that ultimately produced today's many types of garden roses.

There are several major classes of garden roses. The best-known and most popular class of rose is the hybrid tea roses, which account for the majority of roses grown in greenhouses and gardens and sold in florist shops. Hybrid teas come in the complete range of rose colours and have large, symmetrical blossoms. Hybrid teas resulted from the crossbreeding of frequently blooming but fragile tea roses with vigorous hybrid perpetual roses. The hybrid perpetuals achieved great popularity until they were supplanted by the hybrid teas in the early 20th century. Polyantha roses are a class of very hardy

roses that produce dense bunches of tiny blossoms. Floribunda roses are hardy hybrids that resulted from crossing hybrid teas with polyanthas. Grandiflora roses are relatively new hybrids resulting from the crossbreeding of hybrid teas and floribunda roses. Grandifloras produce full-blossomed flowers growing on tall, hardy bushes. Among the other classes of modern roses are climbing roses, whose slender stems can be trained to ascend trellises; while shrub roses, which develop into large bushes; and miniature roses, which are pygmy-sized plants bearing tiny blossoms. Altogether there are approximately 13,000 identifiable varieties of roses in these and other classes.

No other flower is so universally known and admired as that of the rose. Its blossoms range in colour from white through various tones of yellow and pink to dark crimson and maroon. Many varieties have been bred with beautiful blends of colour. Rose flowers' size ranges from tiny miniatures 1.25 cm (0.5 inch) in diameter to flowers measuring more than 17.5 cm (7 inches) across. Roses have a delightful fragrance, which varies according to the variety and to climatic conditions.

India has a long tradition of floriculture. It is recognized as a lucrative business since it has higher potential per unit area than most of the field crops, and even horticultural crops both for domestic and export market. Flowers make the environment happy, clean and pollution free. They are also used for decorations and aesthetic purposes; they have

tremendous economic value as a cut flowers, loose flowers, for perfumes and other products, which play a major role in uplifting our national income.

Rose is world's leading cut flower in production as well as in market. It shares about 24% of the world trade of cut flowers. India is the second largest flower grower in the world. Considering increasing scope and importance of floriculture, Government of India has launched massive programme to achieve export target. Presently in India total area under floriculture is about 2 lakh 33 thousands ha. with a production of loose flowers 1729.2 thousands MT and cut flowers 76731.9 lakh nos. (Anonymous, 2013). In Maharashtra state total area under floriculture is 22,000 ha. with a total production of loose flowers 119 thousands MT (6.88%) and cut flowers 7914.0 in lakh nos.(10.3%) (Anonymous, 2013).

The country has strength for development of this industry as has an availability of different climatic zones, good climate and soil, cheap labour, enough land and skilled manpower. Though sector have not organized to the level of systematic cultivation and knowledge about post harvest activities but past experiences and quest to achieve excellence have turned the sector to be competitive and now keeping pace with global brand, India is venturing into commerce and online marketing of flowers to global buyers.

The major centers for flower marketing are metropolitan cities like Mumbai, Kolkata, Chennai, Bangalore, Delhi in

India and Pune, Mumbai, Nasik, Ahmednagar, Sangli, Kolhapur, Thane, Satara and Nagpur in Maharashtra.

Now-a-days, roses are available in numerous attractive colours and shades. All present day colourful roses are the result of extensive hybridization, spontaneous and induced mutations and selections.

For a modern and industrialized horticulture, there is always demand and necessity of new varieties. To develop new varieties through genetic manipulation, there are several plant breeding techniques. Through cross breeding technique, the main attempt of plant breeder is to combine the beneficial characters from different sources into one genotype. From such pooled genotypes, sometimes it is possible to select directly a particular genotype which is superior to the existing cultivar. Mutation breeding on the other hand, is an established method for plant improvement. By this method plant genes are altered by treating seeds or other plant parts to chemical or physical mutagens.

Mutation breeding has been most successful in roses in inducing novelties. The possibilities for creating different forms and improving roses are infinite , and breeder will always have future goals to work towards it. Therefore the present investigation on Mutation Breeding in Rose (*Rosa indica* L.) was planned and conducted at Modibaug, College of Agriculture, Pune with the following objectives :

1] To study the morphological changes in rose as a result of chemical mutagenesis.

2] To study chimeric growth pattern.

2. REVIEW OF LITERATURE

Mutation breeding is considered as one of the important methods to generate additional useful variability in crop plants including vegetatively propagated horticultural crops.

The term mutation, coined by Hugo de Varies in 1900, is the sudden heritable change in the genetic constitution of an organism and becomes the basis for variation in characters. Artificial induction of mutation offers a greater scope for crop improvement (Gustafsson, 1947). It brings beneficial changes in single character without altering linkages, in diploidizing of artificial polyploids and generating variability.

From the discoveries made by Muller (1927) and Stadler (1928) indicated that X-ray could modify the genetic constitution of Drosophila and maize marked the onset of mutation in crop improvement. Likewise, in barley crop, some economic mutants are the resultant of mutation breeding which gave an impetus to the research workers and a large number of crop plants including ornamental flower crops are subjected to mutation.

Chan (1966) irradiated five rose cultivars (Peace, Queen Elizabeth, Better Times, Baccara and Tropica) with 7-8 Krad or higher doses of X-ray. He detected a number of mutations affecting flower colour and growth habit.

Heslot (1966, 1968) tried to induce mutations through gamma irradiation (4 or 8 Krad) and EMS (8ppm) in diploid, triploid and tetraploid cultivars and detected a wide range of mutations in flower colour. High frequencies of mutations in cultivars Orange Triumph and Gloria, Mundi could be explained as consequences of pre-existing periclinal chimerism.

Nakajima (1970) reported irradiated plants were repeatedly cutback in order to force latent buds into growth and thus encourage the production of mutant tissue. The best results were obtained by irradiating in late April-late May at about 10 Krad for up to 10 days. There were marked differences in response between cvs Crimson Glory and Golden Masterpiece rarely mutated whereas many sports were obtained in Peace, Queen Elizabeth and Kordes Perfecta.

Gupta and Shukla (1970) observed that gamma ray treatment of Montezuma and Super star rose budwood at 4, 5 and 6 Krad before budding generally led to reductions in bud-take, plant survival and plant height. Somatic mutations were induced in flower colour and shape in Montezuma only, 5 Krad being the most effective dose. Super star was the more radiosensitive of the two cultivars.

Gupta and Shukla (1971) studied the effects of gamma irradiation on some scented roses. Budwood of twelve scented cultivars of garden roses was irradiated with 4 kR of gamma rays before budding the eyes on Edward rootstock. Percentage of bud-take and survival, height of plants and

number of flowers per plant decreased and percentage of abnormal flowers increased after irradiation. Somatic mutations were induced in flower colour in Bettina, Lady Florence Stronge, McGredy' Sunset and President Poincare.s

Kaicker and Swarup (1972) reported induced mutant in rose by using gamma rays as a mutagenic agent. Cv. "Pusa Christina", a pink coloured mutant was developed from cv. 'Christian Dior' by using gamma irradiation. Another variety named "Abhisarika" with pink strips was developed from a 'Kiss of fire' by using gamma irradiation.

Dommergues *et al*. (1976) made a comparative study of effects of both physical (gamma rays) and chemical (Ethyl methane sulphonate - EMS) mutagens among diploid (Gloria, Mundi Border King), triploid (Orange Triumph) and tetraploid (Peace and several others) cultivars. One year old plants were treated with 8-9 Krad gamma rays and 0.5-0.8% EMS for 24 hours at 20^0C. Differential sensitivity to both the mutagens were recorded among the cultivars and radiation was found to be more effective in inducing mutation especially in polyploids. Dommergues (1976) also studied *in vitro* induced mutagenesis in roses.

Kaicker and Swarup (1978) reported induced mutation in rose cv.Gulzar by using chemical mutagens (EMS 0.25%).

Lata (1980) studied the effect of ionizing radiation on roses. Bud-wood from seven rose cultivars exhibiting five different colors were exposed to 0, 3, 4 and 5 krad of gamma rays. A similar response was observed for all exposed cultivars; it included dose response reductions in

bud take, number and height of shoots, survival, flowers, petal weight and pollen fertility. The results suggest that the floribunda rose, i.e. Pink Parfait was more suitable for induction of mutations as compared with the six Hybrid Teas.

Gupta and Datta (1982) observed reaction in sprouting of bud, survival of plant and number flowers per plant when eye bud of rose cv. “Junier miss” and *Rosa damascana* were irradiated with 3 to 5kr gamma rays. Eye bud were budded on the rootstock *Rosa indica var.odorata.*

Datta and Gupta (1982c) used 3kr dose of gamma rays to cv.”America’s Junier miss”. They found white coloured mutant in rose and later they named it as “Sukumari”. Flower diameter and size of petal was significantly reduced. High pollen sterility accompanied with reduced flower size was also quite significant.

James (1983) mentioned in his article the potentiality of mutation breeding for developing new roses. He initiated mutation breeding work thirty years ago after the World WarII; radioactive isotopes were readily available (personal communication, American Rose Magazine, Dec. 1961). He irradiated terminal buds of roses with Cobalt-60 and from this experiment, and developed two mutations. One mutant developed from rose cv. Queen Elizabeth was named as Paula and the other mutant was named as Pink Hat which was developed from an unnamed floribunda.

Datta and Gupta (1983a) irradiated budwood of five rose cultivars (Eiffel Tower, First Prize, Pink Parfait, Queen

Elizabeth, Super Star) with 3, 4 and 5 Krad of gamma rays. They observed reduction in sprouting, survival, plant height, and number of leaflets. Various types of leaf abnormalities were recorded. Somatic mutation in flower colour was induced in the cultivar Queen Elizabeth.

Datta and Gupta (1983b) developed yellow flower colour mutant after irradiating budwood of rose cv. Contempo (orange with yellow eye) with 3 Krad of gamma rays. Datta (1985) observed differential radio sensitivity with respect to sprouting, survival and plant height in a radiation breeding programme with nine garden roses. The frequency of mutation varied with the cultivar and dose of gamma rays.

Datta (1986a) irradiated budwood of two miniature roses (Magic Carrousel and Windy City) with 3, 4 and 5 Krad of gamma rays. Reduction in sprouting, survival, plant height and number of flowers and various types of leaf abnormalities were recorded. Somatic mutations in flower colour were induced in both the cultivars. The original colour of Magic Carrousel is white with red edges, while in the mutant no red colour developed at the edges of petals. The colour of Windy City is deep pink and the mutant colour is very light pink.

Datta (1986b) developed mutant in rose cv. Imperator after gamma irradiation where bunch of flowers develop at the centre of each flower.

Khalatkar (1986) recommended treatment of auxiliary buds with different concentrations of colchicines or

chemical mutagens (EMS, Sodium azide) for rose improvement programme specially for creating new rose.

Kaicker (1987) isolated solid colour mutants in rose cultivar Folklore after irradiation and treatment with chemical mutagen followed by *in vitro* propagation.

Datta (1988c, 1989d, 1992b) irradiated budwood of rose cv. Mrinalini (Phlox pink) with 3-5 Krad of gamma rays. Data were recorded on sprouting, survival, plant height, morphological abnormalities and induction of mutations in flower colour. In the first year, no mutation could be detected. One branch of plant in 3 Krad treated population showed mutation in flower colour in the second year after drastic pruning. The mutant flower colour was light pink (Blossom pink).

Datta (1993a) isolated pink flower colour mutant after irradiating budwood of rose cultivar Zorina (Grenadine Red) with gamma rays.

Datta (1994a) developed creamish yellow flower colour mutation in the rose cv. Salmon Beauty (Salmon colour) by treating budwood with 4 Krad gamma rays.

Murugesan *et al.* (1993) made an attempt to induce mutations in rose cv. ST. Boniface by tuber extract of *Gloriosa superba*. Mutations were induced for stalk length, flower diameter, petal size and number.

Yamaguchi *et al.* (2003) studied the effects of mutation induction by ion beam irradiation on axillary buds in rose. Axillary buds were irradiated with carbon and helium ion beams, and the solid mutants emerged after irradiation by

repeated cutting back. Irradiation with both ion beams induced mutants in the number of petals, in flower size, in flower shape and in flower color in each cultivar.

Essential oil bearing roses

Mutation experiment was also carried out with essential oil roses.

Arinshtein and Krapivenko (1980) injected shoots with N-nitroso-N-ethyl urea (NEU) repeatedly and dechimerised the shoots by cutting back twice. They detected mutations that were not commercial ones.

Gupta *et al.* (1982) irradiated cuttings of *Rosa damascena* with 0, 1 and 2 Krad of gamma rays. Reduction in sprouting, sprout number and plant height was recorded after irradiation. Somatic flower colour mutation induced after irradiation was isolated in pure form.

Recurrent irradiation

Recurrent gamma irradiation showed cumulative effects on sprouting, survival and plant height. Percentage of somatic mutation and spectrum of mutation were higher after recurrent irradiation in comparison to single irradiation in rose cv. Contempo (Datta 1986a, 1993). Reduction in sprouting, survival and plant height after exposure to gamma rays have been attributed to non-heritable physiological disturbances of growth substances.

Kaicker and Kumar (1992) observed drastic change in plant height of some induced mutants of Folklore after recurrent gamma irradiation. Comparative study of the two dwarf mutants, namely, 'Dwarf Striped Pink' and 'Dwarf

Bright Yellow' revealed that there were marked differences in terms of morphological, internode length, peduncle length, number of petals, petal area and flower colour between dwarf mutant and the original rose cv. Folklore.

Colchicine treatment

Colchicine has been used for a long time as a polyploidizing agent. It has been used successfully to produce polyploids for cytogenetic research and for breeding programme in many plant species. Induction of mutations by colchicines in several crop plants has been reported by many workers.

Gupta and Datta (1983a) treated budwood of rose cv. Contempo (orange with yellow eye) with 0.0625 and 0.125% colchicines for 4 hours. Reduction in sprouting, survival, sprout height and branch and flower number was recorded after colchicines treatment. Somatic mutations in flower colour were induced. The mutant colour were Tangerine Orange and Empire Yellow.

Datta and Gupta (1985b) removed eyes from budwood of rose cv. Contempo and treated with 0.0625 and 0.125% aqueous solution of colchicine for 4 hours. Delay and decrease in sprouting and survival were recorded after treatment with higher concentration of colchicine. Various types of leaf abnormalities were recorded. Somatic mutation in flower colour was detected in 0.0625% treated population. Here also the mutant colour was Yellow. Higher percentage of sprouting and survival was recorded after treatment with 0.0625% colchicines.

Datta (1988e, 1989a,b,c) reported effects of gamma rays and colchicines on garden rose. Reports clearly indicate that colchicines can be successfully used for inducing somatic flower colour mutations. It has been pointed out that normally after colchicine treatment attention is paid to chromosome duplications and its effect on phenotype. When there is no polyploidy formation and when there is no gigantism in desired characters in induced polyploidy in particular taxa, colchicine breeding is thought to be unsuccessful. But careful observstions have led to the understanding that although colchicine is known more familiarly as a polyploidizing agent, it may also be used as a very good mutagen.

Datta (1994c) successfully used colchicines for the first time to induce flower colour mutation in rose cv. Contempo. Studies clearly indicated that colchicine induces similar mutagenic effects on budwood of rose as those produced by gamma rays and other mutagens.

3. MATERIAL AND METHODS

The present study on "Mutation Breeding in Rose (*Rosa indica* L.)" was conducted at College of Horticulture Pune, MPKV Rahuri, during 2013-2014. During the course of investigation the materials used and techniques adopted are given below.

3.1 Details of experimental material

3.1.1 Location

Geographically, Pune is situated at 18°- 32' North latitude and 73°- 51' East longitude at 569 meters above sea level on Deccan plateau at the confluence of Mula and Mutha rivers. It is second largest city of Maharashtra and is considered the state's cultural capital.

3.1.2 Experimental site

The experiment was laid out at the Hi-Tech floriculture and vegetable project, College of Agriculture, Pune-5.

3.1.3 Climate and weather condition

The average maximum and minimum temperature recorded during the period of experiment was 37.8^{0}C and 7.5^{0}C, respectively while, average maximum and minimum relative humidity was recorded 96 and 21 per cent, respectively. The data regarding meteorological parameters prevailing at Pune during the course of investigation has presented in Appendix.

3.1.5 Planting materials

Rose cultivar Gladiator was selected for the present experiment. Rose is commercially propogated by T-budding. Scions of about 10-12 cm in length having 4-5 healthy buds

from one year old growth were selected for mutagenic treatment.

3.1.6 Source of planting material

Scions of Rose cultivar Gladiator were obtained from farmer Shri Vinayak Nimhan, Pune (M.S.)

3.2 Method

3.2.1 Treatment with chemical mutagens

Well developed buds were treated by dipping the scion wood with buds in various concentrations of EMS and MMS (0.20%, 0.25%, 0.30%, 0.35%) respectively for 8 hours. The treated and control eye buds were budded on the same day on root stock *Rosa multiflora*.

3.2.2 Experimental details

The details of the experiment are given below:

- **Name of the crop** **:** Rose (*Rosa indica)*
- **Variety** **:** Gladiator
- **Growing media** **:** Coco peat
- **Design** **:** Completely Randomized Design (CRD)
- **Number of treatments** **:** 9
- **Number of replications** **:** 4
- **Number of plants/treatment** **:** 20

Treatments :

Chemical Mutagens :

1. EMS 0.20%
2. EMS 0.25%
3. EMS 0.30%
4. EMS 0.35%

5. MMS 0.20%
6. MMS 0.25%
7. MMS 0.30%
8. MMS 0.35%
9. Control

Treatment Details :

Sr.No.	Treatment code	Chemical
1	**T_1**	**EMS 0.20%**
2	**T_2**	**EMS 0.25%**
3	**T_3**	**EMS 0.30%**
4	**T_4**	**EMS 0.35%**
5	**T_5**	**MMS 0.20%**
6	**T_6**	**MMS 0.25%**
7	**T_7**	**MMS 0.30%**
8	**T_8**	**MMS 0.35%**
9	**T_9**	**Control**

3.2.3 Cultivation details

The details of cultural operations carried out during the course of investigation are as follows:

3.2.4 Media preparation and transplanting

Budded plants are kept in nursery till they sprout. The sprouted grafts were transplanted into pots in cocopeat. Before transplanting cocopeat was washed thoroughly with tap water and CaN for nourishing and kept overnight covered by polythene paper. Coir was placed at the bottom of the pot and then filled with cocopeat. Weeding was done whenever necessary.

3.3 Observations

The following observations were recorded to determine the effects of chemical mutagens on rose. Each and every plant per treatment per replication was observed for morphological characters. Any variation or abnormality in different treatments was also recorded.

3.3.1 Growth Observations

3.3.1.1 Number of Days required for sprouting

Number of Days required for sprouting of buds from the day of budding was recorded. Keeping this in view, stimulation for early sprouting and delayed sprouting was determined in each treatment.

3.3.1.2 Plant height at the time of flowering

The height of each plant was recorded in centimeter (cm) with help of measuring steel tape from the budded portion up to the growing point at the time of flowering. The mean height of the plant was calculated from the same.

3.3.1.3 Plant spread at the time of flowering

The plant spread of each plant was measured at two positions in the North-South and the East-West directions at right angles to each other at the time of flowering stretching a meter scale and expressed in centimeters (cm). The mean of these observations were taken for calculating the plant spread in the North-South and the East-West directions.

3.3.1.4 Leaf area

The leaf area of three leaves of each plant was measured at the time of flowering by graph paper. The mean was worked out and expressed in square centimeters (cm^2).

3.3.1.5 Number of thorns on flower stalk

The number of thorns on flower stalk from peduncle to the downward side up to 25 cm were counted visually for five flower stalks of each plant and from this the mean number of thorns on flower stalk was worked out.

3.3.2 Floral Characters

3.3.2.1 Days to first flower bud initiation

The number of days required for first flower bud initiation was counted from the date of bud sprouting of each plant in every treatment.

3.3.2.2 Average Weight of flower

Five fully opened flowers from each plant were cut from peduncle and their weight was recorded on weighing balance, mean was worked out and expressed in grams (g).

3.3.2.3 Colour of flower

The colour of the fully opened flower was recorded before they started fading by comparing their colour with colour shades mentioned in Royal Horticultural society's colour chart.

3.3.2.4 Number of petals/flower

Number of petals were counted for five flower of each plant and average was worked out for single plant.

3.3.2.5 Length of peduncle (cm)

The length of peduncle measured with help of measuring scale for five flowers for each plant and the mean was taken as length of peduncle.

3.3.2.6 Vase life (days)

The rose flower stalks were harvested having 25cm stalk length with the help of secateur at tight bud stage. Two stalks from each plant were placed in flasks containing 150 ml of distilled water. It was recorded in days and mean was calculated

3.3.2.7 Petal area (cm^2)

The area of single petal was recorded on area meter and expressed in sq. centimeters (cm^2).

3.4 Statistical analysis

The recorded data on various observations during the course of investigation were statistically analyzed using Completely Randomized Design as suggested by Panse and Sukhatme (1967). The analysis was carried out at college computer section of Agricultural Statistics. The appropriate standard error of mean (S.E.m.) and the Critical Difference (C.D.) were calculated at 5% level of probability. Data have been depicted by suitable graphs, figures and the appropriate tables.

4. EXPERIMENTAL RESULTS

The present investigation entitled “Mutation breeding in rose (*Rosa indica* L.) was conducted at College of Horticulture Pune. The observations recorded during investigation were analyzed statistically for its significance and are presented in this chapter under appropriate headings:

4.1 Observations on growth characters

4.2 Observations on flowering characters

4.1 Observations on growth characters

4.1.1 Days required for bud sprouting

The data pertaining to number of days required for sprouting as influenced by chemical mutagen treatments are presented in Table.2 and graphically depicted in Fig.1.

The effect of chemical mutagen treatments was non-significant with respect to number of days required for sprouting. Treatment T_9 (control) required minimum days for sprouting (24.85) after budding and treatment T_4 which with was highest concentration of EMS (EMS 0.35%) required maximum number of days for sprouting (31.97) while treatments T_1, T_2 , T_3, T_5, T_6, T_7 and T_8 recorded 27.50, 29.30, 30.63, 28.15, 28.28, 28.31 and 30.79 days for sprouting respectively. Sprouting was delayed with the increasing concentration of EMS and MMS.

4.1.2 Plant height at the time of flowering

Plant height is very important character for growth as well as for the quality of flowers. The data pertaining to plant height at the time of flowering which was influenced

by chemical mutagen treatments are presented in Table.2 and graphically depicted in Fig.2.

The significant variations were observed in height of plants due to effects of different treatments of chemical mutagens. The treatment T_9 (control) had maximum plant height (33.05 cm) and it was at par with treatment T_1 (29.59), T_5 (30.40 cm) and T_6 (29.90). Maximum reduction in plant height (25.25 cm) was noted in treatment T_4 (EMS 0.35%). The trend observed with various treatments was decrease in plant height with increase in concentration of mutagens.

Table-2 Effect of chemical mutagens on growth characters.

Sr. No.	Treatments	Days required for bud sprouting	Plant height (cm)	Plant spread (cm)	
				E-W	N-S
1	T_1 (EMS 0.20%)	27.50	29.59	20.50	22.16
2	T_2 (EMS 0.25%)	29.30	29.13	20.24	21.65
3	T_3 (EMS 0.30%)	30.63	28.32	19.39	20.93
4	T_4 (EMS 0.35%)	31.97	25.25	19.38	16.65
5	T_5 (MMS 0.20%)	28.15	30.40	19.94	21.25
6	T_6 (MMS 0.25%)	28.28	29.90	19.73	20.42
7	T_7 (MMS 0.30%)	28.31	28.98	18.71	19.91
8	T_8 (MMS 0.35%)	30.79	28.68	18.58	19.02
9	T_9 (Control)	24.85	33.05	19.99	20.79
S. E. ±		2.0735	1.3001	0.3763	0.9065
C. D. at 5 %		NS	3.7724	1.0919	2.6303

4.1.3 Plant spread (cm)

The data pertaining to plant spread at the time of flowering as influenced by chemical mutagen treatments are presented in Table.2 and graphically depicted in Fig.3 and 4.

Data revealed that the East-West and the North-South plant spread varied significantly with the treatments of chemical mutagens. Maximum East-West and North-South plant spread (20.50 cm and 22.16 cm respectively) was recorded with the minimum concentration of EMS (0.20%). The East-West spread was found minimum for treatment T_8 (18.58 cm) and North-South plant spread was found minimum for treatment T_4 (16.65 cm). Decreased plant spread with increasing concentration of EMS and MMS was recorded.

4.1.4 Leaf area (cm^2)

The data pertaining to leaf area at the time of flowering influenced by chemical mutagen treatments are presented in Table.3 and graphically depicted in Fig.5.

It can be seen from the data presented in Table 3 that the different treatments had significant influence on leaf area. The treatment T_1 (EMS 0.20%) recorded significantly maximum leaf area at the time of flowering (46.53 cm^2) which was at par with treatment T_2 (40.31 cm^2). In the treatment T_9 (control) minimum leaf area (24.08 cm^2) was recorded. Reduction in leaf area was recorded with the increasing concentration of EMS and MMS.

Table 3 Effect of chemical mutagens on growth characters.

Sr. No.	Treatments	Leaf area at the time of flowering	Number of thorns on flower stalk
1	T_1 (EMS 0.20%)	46.53	15.04
2	T_2 (EMS 0.25%)	40.31	13.07
3	T_3 (EMS 0.30%)	35.77	10.99
4	T_4 (EMS 0.35%)	32.77	10.59
5	T_5 (MMS 0.20%)	33.64	17.63
6	T_6 (MMS 0.25%)	32.69	13.73
7	T_7 (MMS 0.30%)	31.21	13.73
8	T_8 (MMS 0.35%)	29.32	12.77
9	T_9 (Control)	24.08	14.04
S. E. ±		2.6431	1.1954
C. D. at 5 %		7.6695	3.4688

4.1.5 Number of thorns on flower stalk

The data regarding the effect of different chemical mutagens on number of thorns on flower stalk presented in Table 3 and graphically depicted in Fig. 6.

The significant variations were observed in number of thorns on flower stalk. The treatment T_5 (MMS 0.20%) had maximum thorns (17.63) which was at par with treatment T_1 i.e. EMS 0.20% (15.04). The significant reduction was found in treatment T_4 EMS 0.35% which was highest concentration of EMS (10.59)

4.2. Flowering characters

The data in respect of flowering characters such as days required for first flower bud initiation, average weight

of flower, number of petals per flower, length of peduncle, flower colour, vase life and petal area are presented in table 4 and table 5.

Table 4. Effect of chemical mutagens on flowering characters.

Sr. No.	Treatments	Days required for first flower bud initiation	Average weight of flower	Number of petals per flower	Length of peduncle
1	T_1 (EMS 0.20%)	23.63	7.47	84.21	7.31
2	T_2 (EMS 0.25%)	25.21	7.43	81.91	7.15
3	T_3 (EMS 0.30%)	25.68	6.69	76.21	5.87
4	T_4 (EMS 0.35%)	26.71	5.74	67.58	5.10
5	T_5 (MMS 0.20%)	23.39	6.17	70.48	6.16
6	T_6 (MMS 0.25%)	24.96	5.98	70.58	6.06
7	T_7 (MMS 0.30%)	26.79	5.38	63.65	6.01
8	T_8 (MMS 0.35%)	27.51	5.22	62.32	5.84
9	T_9 (Control)	22.85	5.09	61.59	5.75
S. E. ±		1.0157	0.3181	2.4841	0.3641
C. D. at 5 %		2.9473	0.9231	7.2083	1.0566

4.2.1. Days required for first flower bud initiation

The data regarding observation on number of days required for first flower bud initiation are presented in Table 4 and graphically illustrated in Fig. 7.

Significantly earlier flower bud initiation was observed (22.85 days) with T_9 (control) and it was at par with treatments T_1 (23.63 days), T_2 (25.21 days), T_3 (25.68 days), T_5 (23.39 days) and T_6 (24.96 days). The treatment T_8 (MMS

0.35%) required maximum (27.51 days) for first flower bud initiation than all the other treatments.

4.2.2. Average weight of flower

The data on average weight of flowers along with peduncle as affected by chemical mutagens are presented in Table.4 while graphically represented in Fig.8.

It can be seen from the data presented in Table 4 that the different treatments had significant influence on average weight of flowers. The maximum average weight of flowers was noted in treatment T_1 (7.47 g) which was at par with treatment T_2 (7.43 g) and T_3 (6.69 g). The minimum average weight of flowers was recorded in treatment T_9 control (5.09 g). Reduction in flower weight was recorded with the increasing concentrations of EMS and MMS chemical mutagens.

4.2.3. Number of petals per flower

The data in respect to number of petals per flower are depicted in Table.4 and graphically presented in Fig.-9.

The significant variations were observed in number of petals per flower. The treatment T_1 EMS 0.20% had maximum number of petals per flower (84.21) and it was at par with the treatment T_2 i.e. EMS 0.25% (81.91). The minimum number of petals was found in treatment T_9 control (61.59). Number of petals per flower was reduced after treating with the increasing concentrations of EMS and MMS.

4.2.4. Length of peduncle

The data regarding difference in length of peduncle (cm) in rose due to chemical mutagens are presented in Table 3 and graphically shown in Fig. 10.

The length of peduncle was significantly affected by the different concentrations of chemical mutagens. The length of peduncle was significantly increased in treatment T_1 (7.31 cm) that was at par with treatment T_2 (7.15 cm). Peduncle length was reduced in treatment T_4 having minimum length of peduncle (5.10 cm).

Table 5. Effect of chemical mutagens on flowering characters.

Sr. No.	Treatment	Colour of flower	Vase life (Days)	Petal area (cm^2)
1	T_1 (EMS 0.20%)	Rose red	9.15	20.24
2	T_2 (EMS 0.25%)	Rose red	8.20	19.88
3	T_3 (EMS 0.30%)	Rose red	7.94	19.81
4	T_4 (EMS 0.35%)	Rose red	7.74	19.59
5	T_5 (MMS 0.20%)	Rose red	8.11	20.10
6	T_6 (MMS 0.25%)	Rose red	7.94	20.04
7	T_7 (MMS 0.30%)	Rose red	7.93	19.92
8	T_8 (MMS 0.35%)	Rose red	7.87	19.82
9	T_9 (Control)	Rose red	7.55	19.50
S. E. ±		-	0.1709	0.1445
C. D. at 5 %		-	0.4958	0.4192

4.2.5 Colour of flower

The petal colour of fully opened flower was recorded by comparing their colour with colour shades mentioned in Royal Horticultural Society's colour chart.

The data presented in Table 5 revealed that the treatments of different concentrations of EMS and MMS did not show any variation in petal colour of flowers of treated and untreated plants. The petal colour of treated and untreated (control) was Rose Red.

4.2.6 Vase life

The observations regarding the vase life as influenced by chemical mutagens are presented in Table.5 and graphically represented by Fig.11.

The vase life was significantly affected due to chemical mutagens treatments. Significant maximum days of vase life was recorded in treatment T_1 (9.15 days) which was followed by T_2 (8.20 days) and T_5 (8.11 days). The minimum vase life was noted in treatment T_9 control (7.55 days).

4.2.7 Petal area (cm^2)

The data in respect to petal area are depicted in Table.5 and graphically presented in Fig.12.

The significant variations were observed in petal area of flower. The treatment T_1 (EMS 0.20%) had maximum petal area (20.24 cm^2) and was statistically at par with the treatments T_2 EMS 0.25% (19.88 cm^2), T_5 MMS 0.20% (20.10 cm^2), T_6 MMS 0.25% (20.04 cm^2) and T_7 MMS 0.30% (19.92 cm^2). The minimum petal area was found in treatment T_9 control (19.50 cm^2).

5. DISCUSSION

Induced somatic mutations play an important role as a means of crop improvement (Hugo de Vries 1900, Muller 1927, Stadler 1928, Gustafson 1947, Sheehan and Sagawa 1959 and Sozonova and Syrovatka 1974) in ornamental crops. It showed rich variation of colours, more number of petals, slow opening of bud and longer vase life which are the main reasons for its ever increasing demand. Induced mutations assume special significance *i.e.* practical and scientific in the improvement of roses. Besides this, in ornamental crops like rose, any change which gives novel flower colour and form becomes more acceptable to the growers. Breeding although provides thrilling results in rose but is time consuming where as mutation breeding takes about three times less period and produces even unusual types which cannot be obtained ordinarily through conventional methods. Mutation breeding has been most successful in roses in inducing novelties. The possibilities for creating different forms and improving roses are infinite, and the breeder will always have future goals to work towards it. The objectives of the present study were to study the effect of chemical mutagens on growth and flowering characters of rose.

5.1Observations on growth characters

5.1.1 Days required for bud sprouting

Non-significant effect of chemical mutagen treatments was observed on days required for bud sprouting, in which

sprouting of buds was enhanced at lower concentrations while it was delayed at higher concentrations. Treated buds at higher concentrations of both EMS and MMS took longer time to sprout. Minimum (24.85) days for sprouting were recorded in treatment (T_9) control. It is revealed from the results that higher concentrations of chemical mutagens suppressed sprouting and on the other hand time taken for sprouting was increased. This may be due to inactivation of auxins and biological effect of alkylation of EMS and MMS. Treatment T_4 (EMS 0.35%) recorded maximum number of days required for sprouting than all other treatments. This finding was supported by Kaicker and Swarup (1972), where they found that with the increase in concentration of chemical mutagens the sprouting was delayed.

5.1.2 Plant height

The plant height differed significantly in various treatments. Treatment T_9 (control) recorded maximum plant height (33.05 cm) while treatment T_4 which was the highest concentration of EMS (EMS 0.35%) recorded minimum plant height (25.25 cm). Reduction in plant height was observed with the increasing concentration of EMS and MMS and it was more in EMS. The present findings are in conformity with the work of Gupta and Shukla (1971), where they observed that with increased radiation in garden roses, decrease in level of auxin and chromosomal damage was responsible for plant height reduction. Reduction in plant height with increase in dose of physical mutagen was also observed by Gupta and Shukla (1970).

5.1.3 Plant spread

Chemical mutagen treatment T_1 (EMS 0.20%) which was the lowest concentration showed significant influence on the East-West and North-South plant spread while other treatments of higher concentrations were found ineffective with decreasing spread of plants in comparison with control. Maximum East-West (20.50cm) and North-South (22.16cm) plant spread was recorded with the minimum concentration of EMS (0.20%). Increase in plant spread in lowest concentration of EMS (0.20%) over control was may be due to stimulatory effect of chemical mutagens. The East-West spread was found minimum in treatment T_8 i.e. MMS 0.35% (18.58 cm) and North-South plant spread was found minimum in treatment T_4 i.e. EMS 0.35% (16.65 cm). The present findings are in agreement with the work reported by Senapati and Raut (2008) in rose cv. First Red.

5.1.4 Leaf area

The treatment T_1 EMS (0.20%) recorded maximum leaf area at the time of flowering (46.53 cm^2) which was significantly superior over other treatments and it was at par with treatment T_2 which had leaf area of 40.31 cm^2. In the treatment T_9 (control) minimum leaf area (24.08 cm^2) was recorded. More increase in leaf area over control was observed in different concentrations of EMS as compared to MMS. It may be because MMS predominantly methylates guanine whereas EMS ethylates guanine and also adenine (Rhaese *et al* 1973). These results are in agreement with those of Senapati and Raut (2008) who reported that

morphological variation between mutant and control plants with regard to leaf size in rosr cv. First Red.

5.1.5 Number of thorns on flower stalk

The significant variations were observed in number of thorns on flower stalk. The treatment T_5 (MMS 0.20%) had maximum thorns (17.63), which was at par with treatment T_1 EMS 0.20% (15.04). The reduction in number of thorns was found in treatment T_4 EMS 0.35% (10.59) and T_3 i.e. EMS 0.30% (10.99). Reduction in number of thorns with increasing concentration of EMS may be due to biological effect of ethylation. Smilansky *et al.* (1986) stated that the appearance of mutations such as thorniness after treating with various doses of gamma rays and EMS was less frequent.

5.2 Observations on flowering characters

5.2.1 Days required for first flower bud initiation

The number of days required for first flower bud emergence was significantly affected by different treatments of chemical mutagens. The treatment T_9 showed early emergence of flower buds. Delay in the emergence of flower bud was recorded with the increasing concentrations of chemical mutagen treatments

This was may be due to in mutation many biosynthetic pathways were altered which were directly and indirectly associated with the flowering physiology (Mahure *et al.,* 2010)

5.2.2 Average weight of flower

The average weight of flower was significantly affected by different treatments of chemical mutagens. The treatment T_1 gave highest weight of flower by 2.38 g more in comparison with control which gave minimum weight of flower. The increase in average weight of flower in lower dose of chemical mutagen was may be due to increase in peduncle length, number of petals per flower and petal area. These results are in agreement with Lata (1980) who found detectable variations in leaf, flower and growth when treated with gamma rays.

5.2.3 Number of petals per flower

The significant variations were observed in number of petals per flower. The treatment T_1 had maximum number of petals per flower (84.21) which was at par with treatment T_2 (81.91). The minimum number of petals were found in treatment T_9 i.e. control (61.59). This variation in number of petals per flower after treating with chemical mutagen was mat be due to alteration in biosynthetic pathways which were directly or indirectly affecting the flowering physiology (Mahure *et al* 2010). These results are in agreement with those of Smilansky *et al.* (1986), Senapati and Raut (2008) and Yamaguchi *et al.*(2003)

5.2.4 Length of peduncle

Peduncle length of flower is an important attribute with regard to market value of rose. Significant differences were observed in peduncle length of flower among various

treatments under study. Reduction in length of peduncle with increase in concentration was recorded. The length of peduncle was significantly increased in treatment T_1 (7.31 cm) which was at par with treatment T_2 (7.15 cm). Peduncle length was reduced at treatment T_4 having minimum length of peduncle (5.10 cm). This may be due to that in chemical mutation phosphate alkylation occurs in addition to base alkylation when treated with alkylating agents like EMS and MMS (Rhaese *et al* 1973). Kaicker and Kumar (1992) also observed change in peduncle length in cv. 'Folklore' when treated with gamma irradiation.

5.2.5 Colour of flower

Colour of flower as a result of effect of chemical mutagens was found to be non significant. There was no variation in colour of flowers obtained from treated plant. This can be attributed to the fact that no chimeric growth was developed in shoot as a result of mutagenesis. Tissues of shoots without chimeric growth lead to non formation of different colour variation in petal. However, Smilansky *et al.*(1986), Gupta and Shukla (1971), Kaicker and Swarup (1972) Gupta and Shukla (1970), Nakajima (1970), Yamaguchi et al (2003) reported colour change in rose after treated with various doses of physical and chemical mutagens.

5.2.6 Vase life

The vase life was significantly affected due to chemical mutagens treatments. The maximum days of vase life was recorded at T_1 treatment i.e. EMS 0.20% (9.15 days). The

minimum vase life was noted in control treatment i.e. (7.55 days). It may be due to increase in number of petals and area of petals which lead to increase in level of sucrose and other soluble sugars and which is responsible for more vase life.

In the present study, the enhanced effect on longevity of the stalk may be due to the positive effect of lower concentration of chemical mutagens on growth hormones.

5.2.7 Petal area

The significant variations were observed in petal area of flower. The treatment T_1 i.e. EMS 0.20% had maximum petal area of flower (20.24 cm^2) followed by T_5 i.e. MMS 0.20% (20.10 cm^2). The minimum petal area was found at treatment T_9 i.e. control (19.50 cm^2). Petal area was reduced with the increasing concentration of EMS and MMS which may be due to inactivation of auxins, chromosomal abberations and biological effect of alkylating agents (EMS and MMS). These results are in agreement with those of Kaicker and Kumar (1992) who also found change in petal area in cv. 'Folklore' as a result of mutation.

6. Summary and Conclusion

The present investigation entitled “Mutation Breeding in Rose (*Rosa indica* L.)” was carried out at College of Horticulture Pune, MPKV Rahuri, during 2013-2014 with the view to find out effect of chemical mutagens on morphological characters of rose. The experiment was laid out in Completely Randomized Design with nine treatments replicated four times. The observations in respect of growth and flowering characters were recorded. The data was statistically analyzed. The results are summarized in this chapter.

The result obtained in respect of growth and flowering characters as influenced by various treatments of chemical mutagens are summarized below.

1. As compared to control, both the chemical mutagens have significant effect on growth and flowering characters but different concentrations of EMS mutagen were found more effective than that of MMS.
2. The effect of chemical mutagens treatments was non-significant with respect to number of days required for sprouting. Sprouting of buds delayed with the increase in concentration of chemical mutagens.
3. The growth characters of plant as represented by height of plant and spread of plant were significantly decreased with the increase in concentration of chemical mutagens.
4. Leaf area was highest at lowest concentration of EMS i.e. 0.20%

5. The significant variations were observed in number of thorns on flower stalk. The treatment T_5 i.e. MMS (0.20%) had maximum thorns (17.63)

6. The period of emergence of first flower bud was significantly delayed with the increase in concentration of chemical mutagens. The earliest emergence of flower bud was observed in the treatment T_9 (control).

7. There was considerable increase in average weight of flowers and number of petals per flower. Highest values for both observations were recorded at treatment T_1 i.e. EMS 0.20%

8. The length of peduncle was highest at the lowest dose of treatment.

9. Vase life and Petal area were highest at lowest concentration of both chemical mutagens.

10. There was no variation found in colour of flower between treated and control plants.

From overall assessment of the results observed in the experiment it can be concluded that treatment (T_1) of EMS 0.20% was found beneficial in relation to growth and flowering of rose (*Rosa indica* L.).

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APPENDIX

Pune Weekly Weather Data 2013

Met. Week	Tmax (°C)	Tmin (°C)	R H I (%)	R H II (%)	RAIN (mm)	R.D (d)	B S S (hr)
1	32.0	15.1	93	27	0.0	0.0	8.9
2	30.1	10.4	95	32	0.0	0.0	8.6
3	31.3	11.5	93	32	0.0	0.0	8.0
4	31.1	11.4	94	27	0.0	0.0	8.5
5	31.7	14.7	82	33	0.0	0.0	7.0
6	31.4	14.7	90	36	0.0	0.0	8.0
7	32.9	15.1	90	26	0.0	0.0	7.9
8	33.5	12.6	85	22	0.0	0.0	9.9
9	34.2	12.4	81	16	0.0	0.0	9.7
10	35.2	14.0	71	19	0.0	0.0	9.5
11	35.6	16.7	73	19	0.4	0.0	8.5
12	35.7	16.1	63	17	0.0	0.0	9.4
13	36.2	17.3	62	19	0.0	0.0	8.5
14	36.8	16.6	58	14	0.0	0.0	9.9
15	38.7	20.1	50	15	0.0	0.0	9.5
16	35.5	19.1	68	22	0.0	0.0	10.8
17	38.4	22.6	55	20	0.0	0.0	10.1
18	39.4	23.8	48	20	0.0	0.0	10.1
19	38.2	23.2	57	22	0.0	0.0	10.1
20	36.7	24.7	66	30	0.0	0.0	8.1
21	36.5	24.9	67	37	0.0	0.0	8.7
22	35.7	24.3	69	40	0.1	0.0	9.0
23	33.1	22.7	84	55	15.6	0.6	4.3
24	28.6	22.8	85	74	15.9	0.6	1.4
25	29.2	22.7	83	69	4.0	0.1	4.5
26	27.8	22.3	88	78	5.2	0.6	1.3
27	28.1	22.3	89	76	2.8	0.3	2.7
28	26.9	21.9	87	79	6.5	0.4	1.6
29	26.2	21.8	91	88	6.5	0.7	0.1
30	25.7	21.7	92	88	11.7	1.0	0.4
31	26.2	21.4	89	86	6.9	0.7	2.4
32	27.7	21.4	86	72	0.6	0.0	3.4

33	28.4	22.0	88	70	0.3	0.0	4.6
34	28.2	21.7	84	67	0.5	0.0	4.0
35	29.1	20.1	87	59	0.1	0.0	5.1
36	29.9	20.4	87	62	2.0	0.3	6.2
37	30.8	21.4	94	62	17.2	0.9	4.3
38	28.8	21.4	88	69	13.8	0.3	3.6
39	28.3	21.1	85	67	1.0	0.1	3.5
40	30.4	21.3	88	60	2.0	0.3	7.2
41	30.0	20.3	86	58	0.0	0.0	7.5
42	32.2	20.2	89	46	2.5	0.1	7.1
43	31.8	19.9	88	49	0.4	0.1	7.5
44	31.8	18.1	87	43	0.0	0.0	8.5
45	30.2	15.3	89	40	0.0	0.0	7.9
46	29.2	12.5	92	36	0.0	0.0	9.0
47	31.2	14.0	92	37	0.0	0.0	9.0
48	29.8	18.5	94	53	2.1	0.1	6.3
49	29.1	13.1	94	36	0.5	0.1	7.8
50	29.3	7.3	94	26	0.0	0.0	9.5
51	29.5	8.5	94	31	0.0	0.0	9.3
52	28.7	12.9	97	42	0.0	0.0	7.5

VITA

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in

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2015

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