

ORIGINAL ARTICLE

Assessment of Mutagenic Population of Tuberose (*Polianthes Tuberosa* L.) Based on Vegetative, Biochemical and Disease Incidence Traits

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ABSTRACT

Mutation breeding appears to be well standardized, efficient and cost-effective breeding techniques that can be exploited for the creation of novel ornamental cultivars. Tuberose (*Polianthes tuberosa* Linn.) is one of the most important bulbous perennial flowering plant, occupies second position after gladiolus in area and production in India. The present investigation was carried at college of Horticulture, Anantharajupeta during 2018-19. Bulbs of Tuberose variety 'Hyderabad Single' were exposed to LD₅₀ dose of gamma rays (20 Gy) at BARC, Mumbai. Each treated bulb was taken as individual treatment (mutant) and observed for various vegetative (Sprouting per cent, Plant height, leaf length, leaf width, plant spread, number of shoots), biochemical (SPAD chlorophyll content) and disease incidence (*Alternaria* leaf spot) characters along with untreated bulbs (control). Among 500 treated bulbs, 265 mutants were survived and these were evaluated and classified based on ranges of vegetative characters, biochemical (SPAD chlorophyll content) and disease incidence (Per cent disease index) characters. Among 265 mutants, M₂, M₇, M₂₁, M₃₂, M₃₃, M₂₁₁ and M₂₃₆ had taken minimum number of days for sprouting (10 DAS), M₂₅₀, M₂₇₆, M₄₁₀ recorded a maximum height of 50.00 cm, M₇ recorded a maximum plant spread of 53.0 cm, maximum leaf length was recorded in M₁₁₆ and M₂₅₀ (49.70 cm), M₁₂, M₃₄, M₃₈, M₆₃, M₉₅, M₁₀₆, M₁₀₇ and M₂₇₆ had recorded maximum leaf width, more number of shoots per clump was recorded in M₂ and M₂₄₈ (20.00), maximum SPAD values was recorded in M₃₀, M₁₁₁, M₁₈₉, M₂₂₂, M₄₂₆, M₄₅₉ (49.8), minimum PDI was recorded in M₂₉, M₆₉, M₈₂, M₁₆₆, M₂₅₇, M₄₀₁, M₄₂₇, M₄₄₀, M₄₄₄ (0), respectively.

Keywords: Tuberose, Vegetative characters, SPAD chlorophyll content, PDI

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INTRODUCTION

Among the tropical ornamental bulbous flowering plants cultivated for production of long lasting flower spikes, Tuberose (*Polianthes tuberosa* L.) occupies a prominent place. It is cultivated commercially for both cut as well as loose flowers and is one of the major bulbous crops. It has pleasant fragrance, long vase-life of spike and wide adaptability to varied climate and soil. The essential oil of Tuberose is one of the most high-priced perfumes [8]. In India, it occupies second position after gladiolus with the total area of 14.92 thousand hectare and production of 106.49 thousand MT loose flowers and 89.83 lakh cut flowers [1-2].

There is constant demand for novelty in existing crops in floriculture industry. So, development of new cultivars through conventional or modern breeding techniques has been a prime objective in commercial

floriculture. Since self-incompatibility exists in tuberose [9] there is limitation of convention breeding methods involving hybridization in it.

Induction of mutations has proven to be sustainable, highly-efficient, environmentally acceptable, flexible, unregulated, nonhazardous and a low-cost technology to enhance crop improvement for the creation of novel ornamental cultivars having aesthetic value and also commercial cultivars having high demand for their flowers and oils extracted from tuberose. Among various mutagens, the gamma irradiations at 1 K rads to 7.5 K rads proved best according to crop response in vegetatively propagated crops [5].

During mutation breeding, to avoid excessive loss of actual experimental materials, radio-sensitivity tests were conducted to determine LD₅₀ doses before massive irradiation of similar material. After arriving LD₅₀ value for gamma rays a huge plant material of tuberose is exposed to LD₅₀ value and further studied for quality traits. Hence the present experiment was conducted to evaluate the mutant population of tuberose based on vegetative, biochemical and disease incidence traits and classified based on ranges.

MATERIAL AND METHODS

The present investigation on 'Assessment of mutagenic population of Tuberose (*Polianthes tuberosa* L.) based on ranges of vegetative, biochemical and disease incidence traits' was conducted at College of Horticulture, Anantharajupeta, Dr. Y.S.R. Horticultural University, Andhra Pradesh during year 2018-19. Bulbs of tuberose var 'Hyderabad Single' was procured from AICRIP on Flower crops, Rajendranagar, Hyderabad. These were irradiated at Babha Atomic Research Centre, Mumbai using physical mutagen, gamma rays at LD₅₀ dose (20 Gy) with untreated as control. The treated bulbs were planted in polybags of size 14x14 inch containing potting media of 2:1:1 ratio of soil, FYM and cocopeat. The experiment containing 265 mutants were evaluated for various vegetative (Sprouting per cent, Plant height, leaf length, leaf width, plant spread, number of shoots), biochemical (SPAD chlorophyll content) and disease incidence (*Alternaria* leaf spot) attributes and classified based on ranges.

RESULTS AND DISCUSSION

The mutants developed from the bulbs of tuberose treated with LD₅₀ value (20Gy) were evaluated for vegetative, biochemical and disease incidence traits and classified based on ranges of attributes.

Vegetative characters

The range for days taken to sprouting varied from 10-185 days after sowing. The mean value for control plants was 33.2. Among the plants whose bulbs were subjected to 20 Gy irradiation, M₂, M₇, M₂₁, M₃₂, M₃₃, M₂₁₁ and M₂₃₆ had taken minimum number of days for sprouting (10 DAS), whereas, M₁₇₈ had taken maximum number of days for sprouting (185 DAS) as given in Appendix-A. Among the irradiated plants 122 mutants (46.03 %) were classified as early, 101 (38.11 %) mutants as medium and 42 (15.84 %) mutants as late (Table 1).

The mean plant height for untreated, control plants was 44.50 cm. Among the plants whose bulbs were subjected to 20 Gy irradiation, M₂₅₀, M₂₇₆, M₄₁₀ recorded a maximum height of 50.00 cm while M₃₀₈ recorded the minimum height of 12.00 cm as given in Appendix-A. The range for plant height varied from less than 40 to more than 50 cm. Among the irradiated plants 125 mutants (47.16 %) were classified as short, 140 mutants as medium tall (52.83 %) and no mutants under range of tall (Table 1).

Alterations in the morphological pattern of any type, through mutagens, are regarded as morphological mutations. Based on segregation pattern of morphological mutants, Reddy and Gupta [7] and Thakur and Sethi [10] observed that most of the true breeding mutants are controlled by single recessive genes, however, Konzak *et al.* [3] argued that the different morphological mutants that bred true in future generations like tall, dwarf, semi dwarf, bushy, prostate and bold seeded mutant types were found to be under the influence of polygenes. Qin *et al.* [6] reported that a dominant dwarf mutation is controlled by a single dominant gene in cow pea.

The general mean value recorded for plant spread in untreated plants was 53.90 cm. Among the mutagenic plants, M₇ recorded a maximum plant spread of 53.0 cm while M₁₇₈ recorded the minimum plant spread of 13.5 cm as given in Appendix-A. The range for plant height varied from 13.5 to 53.0 cm. Among the irradiated plants 19 mutants (7.16 %) were classified as light, 89 mutants (33.58 %) were classified as medium and 157 mutants as heavy spread (59.24 %) (Table 1).

The data recorded for the control mean for leaf length was 44.10 cm. Among the mutagenic population maximum leaf length was recorded in M₁₁₆ and M₂₅₀ (49.70 cm) while the minimum leaf length was recorded in M₃₀₈ (11.60 cm) and it is presented in Appendix-A. The leaf length recorded for irradiated plants ranged from less than 40 cm to more than 50 cm. Based on the range, 149 mutants (56.22 %) were grouped in short, 116 mutants (43.77 %) in medium tall and no mutants under tall group (Table 1).

The range for leaf width varied from less than 2.0 to more than 3.0 cm. The mean value for control plants was 2.10 cm. Among the plants whose bulbs were subjected to 20 Gy irradiation, M₁₂, M₃₄, M₃₈, M₆₃, M₉₅, M₁₀₆, M₁₀₇ and M₂₇₆ had recorded maximum leaf width (2.30 cm), whereas, M₃₀₈ had recorded minimum leaf width (0.70 cm) as given in Appendix-A. Among the irradiated plants, 176 mutants (66.41 %) were classified as narrow, 89 mutants (33.58 %) as medium and no mutants as broad (Table 1).

Table 1 Classification of mutagenic population (265 plants) based on ranges of vegetative, Biochemical and Disease incidence

| S.No | Parameter | Range | Number of putative mutants in each group | Percentage (%) | Control mean |
|-----------|-------------------------------------|-----------|------------------------------------------|----------------|--------------|
| I | Plant growth parameter | | | | |
| 1 | Days taken for sprouting | | | | |
| | Early | 10-68 | 122 | 46.03 | 33.20 |
| | Medium | 68-116 | 101 | 38.11 | |
| | Late | 116-185 | 42 | 15.84 | |
| 2 | Plant height (cm) | | | | |
| | Short | <40 | 125 | 47.16 | 44.50 |
| | Medium tall | 40-50 | 140 | 52.83 | |
| | Tall | >50 | 0.00 | 0.00 | |
| 3 | Plant spread (cm) | | | | |
| | Light | 13.5-26.6 | 19 | 7.16 | 53.90 |
| | Medium | 26.6-39.8 | 89 | 33.58 | |
| | Heavy | 39.8-53 | 157 | 59.24 | |
| 4 | Leaf length (cm) | | | | |
| | Short | <40 | 149 | 56.22 | 44.10 |
| | Medium | 40-50 | 116 | 43.77 | |
| | Long | >50 | 0.00 | 0.00 | |
| 5 | Leaf width (cm) | | | | |
| | Narrow | <2 | 176 | 66.41 | 2.10 |
| | Medium | 2-3 | 89 | 33.58 | |
| | Broad | >3 | 0.00 | 0.00 | |
| 6 | Number of shoots per clump | | | | |
| | Few | 1-7 | 127 | 47.92 | 15.70 |
| | Medium | 7-14 | 118 | 44.52 | |
| | Many | 14-20 | 20 | 7.54 | |
| V | Biochemical traits | | | | |
| 1 | SPAD Chlorophyll content | | | | |
| | Low | 31-37 | 8 | 3.01 | 48.06 |
| | Medium | 37- 43.5 | 98 | 36.98 | |
| | High | 43.5-49.8 | 159 | 60.00 | |
| VI | Disease Index | | | | |
| 1 | Per cent Disease Index (PDI) | | | | |
| | Immune | <5 | 12 | 4.52 | 16.35 |
| | Resistant | 5-10 | 65 | 24.52 | |
| | Moderately Resistant | 11-20 | 161 | 60.75 | |
| | Moderately Susceptible | 21-40 | 26 | 9.81 | |
| | Susceptible | 41-60 | 1 | 0.37 | |

The size of leaves are tightly controlled by environmental and genetic factors that must spatially and temporally coordinate cell expansion and cell cycle activity. The increase and reduction in leaf width might be influenced by physical mutagen on cell activity of plant [4].

The data recorded for the control mean for number of shoots per clump was 15.70. Among the mutagenic population, more number of shoots per clump was recorded in M₂ and M₂₄₈ (20.00) while the less number of shoots per clump was recorded in M₂₉, M₄₉, M₈₂, M₃₇₀ and M₄₃₈ (1.00) and it was presented is Appendix-

G. The number of shoots per clump recorded for irradiated plants ranged from 1.0-20.0. Based on the range, 127 mutants (47.92 %) were grouped under few, 118 mutants (44.52 %) under medium and 20 mutants (7.54 %) under many (Table 1). The variation in number of shoots per clump might be due to the modification of gene expression for the tillering trait depending on the growing condition or mutation induction [11].

Biochemical traits

The maximum SPAD values was recorded in M₃₀, M₁₁₁, M₁₈₉, M₂₂₂, M₄₂₆, M₄₅₉ (49.8) while minimum SPAD values was recorded in M₂₄₄ (31.00) while the untreated control recorded 48.06 as given in Appendix- A. From the data recorded, the range for SPAD values in mutant population ranged from 31 to 49.8. Based on the range plotted, 8 mutants (3.01 %) were classified as low, while 98 mutants (36.98 %) were classified as medium whereas 159 mutants (60 %) were classified as high (Table 1). The variation in SPAD chlorophyll values might be due to occurrence of mutation in chlorophyll synthesis [2].

Disease Incidence

Per cent Disease Index for Alternaria Leaf Spot

The minimum PDI was recorded in M₂₉, M₆₉, M₈₂, M₁₆₆, M₂₅₇, M₄₀₁, M₄₂₇, M₄₄₀, M₄₄₄ (0) and maximum PDI was recorded in M₁₁₇ (41.17%) (Appendix- A). The control plants recorded an average of 16.35 %. A range of less than 5 to 60 was observed in the mutant population. Based on the ranges plotted for mutants, 12 mutants (4.52 %) were grouped under Immune, 65 mutants (24.52 %) were categorised as resistant, 161 mutants (60.75 %) were grouped as moderately resistant, 26 mutants (9.81 %) was classified as moderately susceptible and one mutant (0.37%) was classified under susceptible range (Table 1).

It can be concluded that, among 265 mutants survived after LD 50 treatment, M₂, M₇, M₂₁, M₃₂, M₃₃, M₂₁₁ and M₂₃₆ had taken minimum number of days for sprouting (10 DAS), M₂₅₀, M₂₇₆, M₄₁₀ recorded a maximum height of 50.00 cm, M₇ recorded a maximum plant spread of 53.0 cm, maximum leaf length was recorded in M₁₁₆ and M₂₅₀ (49.70 cm), M₁₂, M₃₄, M₃₈, M₆₃, M₉₅, M₁₀₆, M₁₀₇ and M₂₇₆ had recorded maximum leaf width, more number of shoots per clump was recorded in M₂ and M₂₄₈ (20.00), maximum SPAD values was recorded in M₃₀, M₁₁₁, M₁₈₉, M₂₂₂, M₄₂₆, M₄₅₉ (49.8), minimum PDI was recorded in M₂₉, M₆₉, M₈₂, M₁₆₆, M₂₅₇, M₄₀₁, M₄₂₇, M₄₄₀, M₄₄₄ (0), respectively.

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APPENDIX A: Effect of LD₅₀ on vegetative, biochemical and disease incidence traits in Tuberose (C-CONTROL, M-MUTANT)

| | Days To Sprouting | | Plant Height | | Leaf Length | | Leaf Width | | Number Of Leaves | | Plant Spread | | Number of Shoots Per Clump | | Spad Chlorophyll | | PDI | | |
|---|-------------------|------|--------------|------|-------------|------|------------|------|------------------|------|--------------|------|----------------------------|------|------------------|------|------|------|------|
| | C | M | C | M | C | M | C | M | C | M | C | M | C | M | C | M | C | M | |
| N | 2 | 7 | 12 | 21 | 29 | 30 | 31 | 32 | 34 | 38 | 49 | 63 | 69 | 82 | 95 | 106 | 107 | 111 | 116 |
| C | 10 | 12 | 24 | 13 | 28 | 28 | 10 | 24 | 14 | 28 | 34 | 26 | 36 | 24 | 31 | 26 | 18 | 34 | 28 |
| M | 10 | 10 | 28 | 10 | 175 | 30 | 12 | 10 | 15 | 12 | 30 | 45 | 50 | 19 | 22 | 70 | 94 | 120 | 75 |
| C | 46.0 | 40.7 | 41.5 | 46.3 | 41.3 | 41.4 | 46.2 | 40.2 | 41.5 | 43.5 | 43.6 | 41.0 | 41.3 | 40.0 | 43.6 | 43.6 | 47.0 | 42.5 | 42.6 |
| M | 44.5 | 45.0 | 44.0 | 43.0 | 35.0 | 32.5 | 40.0 | 41.0 | 46.0 | 45.0 | 31.0 | 40.8 | 40.0 | 40.0 | 39.0 | 40.0 | 46.0 | 47.0 | 50.0 |
| C | 46 | 40 | 41 | 46 | 41 | 41 | 46 | 40 | 41 | 43 | 43 | 41 | 41 | 40 | 43 | 43 | 47 | 42 | 42 |
| M | 44 | 44 | 43.5 | 42.5 | 34.5 | 31.5 | 39.6 | 40.5 | 45.3 | 44.5 | 30.8 | 40.2 | 39.5 | 39.6 | 38.7 | 39.6 | 45.5 | 46.5 | 49.7 |
| C | 2.0 | 2.0 | 2.0 | 2.2 | 2.2 | 2.1 | 2.1 | 2.0 | 2.3 | 2.3 | 2.2 | 2.1 | 2.2 | 2.2 | 2.0 | 2.2 | 2.2 | 2.1 | 2.2 |
| M | 2.2 | 2.2 | 2.3 | 2.2 | 2.2 | 2.1 | 2.1 | 2.1 | 2.3 | 2.3 | 2.0 | 2.3 | 2.2 | 2.1 | 2.3 | 2.3 | 2.3 | 1.9 | 2.2 |
| C | 126 | 117 | 115 | 120 | 114 | 117 | 121 | 115 | 118 | 115 | 118 | 124 | 121 | 115 | 115 | 110 | 125 | 121 | 108 |
| M | 110 | 61 | 89 | 78 | 19 | 45 | 50 | 67 | 39 | 48 | 28 | 80 | 32 | 33 | 68 | 62 | 25 | 52 | 102 |
| C | 52.5 | 55.0 | 54.4 | 55.0 | 53.3 | 53.7 | 54.3 | 54.3 | 54.3 | 55.0 | 54.2 | 54.2 | 53.3 | 52.3 | 52.4 | 54.2 | 53.3 | 54.0 | 54.4 |
| M | 52.5 | 53.0 | 48.0 | 47.8 | 41.5 | 31.0 | 49.0 | 50.0 | 49.0 | 47.0 | 41.8 | 44.6 | 32.8 | 33.0 | 44.7 | 42.3 | 41.8 | 42.6 | 45.8 |
| C | 16 | 14 | 14 | 16 | 16 | 16 | 17 | 15 | 15 | 15 | 15 | 15 | 15 | 16 | 15 | 17 | 16 | 16 | 15 |
| M | 20 | 13 | 12 | 15 | 1 | 4 | 11 | 8 | 9 | 10 | 1 | 14 | 3 | 1 | 8 | 14 | 5 | 9 | 10 |
| C | 49.1 | 46.9 | 48.3 | 48.5 | 47.6 | 48.7 | 47.9 | 52.3 | 48.5 | 47.9 | 48.5 | 44.8 | 48.3 | 46.0 | 48.0 | 45.2 | 47.6 | 47.6 | 48.3 |
| M | 45.0 | 42.5 | 40.2 | 40.1 | 47.7 | 49.8 | 48.6 | 46.2 | 40.5 | 47.7 | 46.2 | 39.6 | 45.0 | 40.8 | 41.8 | 48.9 | 48.1 | 49.8 | 43.0 |
| C | 37.7 | 48.5 | 24.1 | 19.2 | 20.4 | 7.1 | 15.0 | 8.0 | 16.1 | 22.2 | 13.0 | 17.7 | 12.5 | 24.4 | 10.9 | 15.4 | 11.8 | 18.6 | 13.2 |
| M | 39.1 | 36.8 | 25.4 | 21.6 | 0.0 | 5.9 | 26.9 | 8.6 | 18.4 | 16.7 | 12.5 | 9.3 | 0.0 | 0.0 | 6.3 | 14.3 | 9.1 | 14.7 | 26.2 |

| | | | | | | | | | | | | | | | | | | | | | |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 41.2 | 0.0 | 16.7 | 8.8 | 8.3 | 10.6 | 21.4 | 8.0 | 16.7 | 28.6 | 0.0 | 12.5 | 9.1 | 14.7 | 0.0 | 7.4 | 9.6 | 0.0 | 17.4 | 0.0 | 0.0 | 10.0 |
| 21.7 | 14.6 | 14.5 | 15.6 | 15.0 | 15.5 | 14.7 | 16.0 | 16.2 | 24.0 | 25.0 | 12.0 | 13.6 | 12.8 | 7.4 | 20.4 | 10.5 | 9.5 | 7.1 | 16.7 | 3.8 | 15.6 |
| 49.7 | 45.6 | 44.0 | 49.8 | 47.6 | 49.8 | 48.7 | 31.0 | 42.9 | 45.2 | 41.7 | 41.9 | 45.6 | 43.3 | 42.5 | 48.3 | 49.8 | 43.9 | 47.0 | 40.0 | 42.3 | 49.8 |
| 47.3 | 48.5 | 48.5 | 47.9 | 47.1 | 47.9 | 47.9 | 48.8 | 47.0 | 48.3 | 44.8 | 50.3 | 45.9 | 49.0 | 48.0 | 50.3 | 47.9 | 49.0 | 48.8 | 48.9 | 44.9 | 47.3 |
| 4 | 2 | 3 | 6 | 14 | 8 | 8 | 14 | 20 | 9 | 3 | 7 | 5 | 1 | 5 | 14 | 6 | 4 | 1 | 7 | 4 | 10 |
| 15 | 17 | 16 | 17 | 16 | 15 | 15 | 16 | 16 | 16 | 16 | 15 | 15 | 16 | 16 | 16 | 16 | 16 | 15 | 15 | 16 | 15 |
| 28.8 | 39.0 | 13.5 | 30.8 | 48.0 | 29.0 | 42.5 | 44.0 | 44.5 | 40.5 | 21.0 | 49.5 | 16.7 | 18.5 | 32.0 | 47.0 | 42.0 | 30.0 | 33.0 | 35.5 | 32.0 | 41.0 |
| 52.4 | 53.3 | 53.3 | 54.8 | 54.4 | 54.3 | 54.4 | 52.4 | 54.7 | 54.3 | 54.0 | 56.9 | 54.4 | 52.3 | 54.3 | 53.3 | 54.4 | 52.3 | 52.4 | 55.0 | 52.0 | 54.9 |
| 21 | 20 | 20 | 52 | 119 | 63 | 92 | 98 | 45 | 54 | 23 | 90 | 30 | 18 | 22 | 144 | 60 | 33 | 25 | 73 | 20 | 63 |
| 110 | 117 | 110 | 121 | 120 | 110 | 110 | 112 | 108 | 106 | 121 | 108 | 121 | 103 | 113 | 125 | 118 | 112 | 110 | 110 | 113 | 123 |
| 1.8 | 1.6 | 1.1 | 1.8 | 1.8 | 1.5 | 1.8 | 2.1 | 1.7 | 2.1 | 1.9 | 2.3 | 0.7 | 1.8 | 2.0 | 1.8 | 1.9 | 2.0 | 2.1 | 1.6 | 2.1 | 2.0 |
| 2.2 | 2.2 | 2.1 | 2.1 | 2.0 | 2.2 | 2.2 | 2.0 | 2.1 | 2.1 | 2.1 | 2.0 | 2.1 | 2.1 | 2.1 | 2.0 | 2.0 | 2.1 | 2.0 | 2.1 | 2.0 | 2.1 |
| 45.3 | 40.5 | 21 | 33.5 | 43.4 | 33.4 | 40.6 | 38 | 39 | 49.7 | 32.1 | 49.4 | 11.6 | 33.6 | 39.5 | 49.6 | 35.6 | 40.6 | 33.5 | 34.5 | 43.7 | 29.5 |
| 41 | 44 | 46 | 41 | 45 | 47 | 42 | 47 | 43 | 45 | 44 | 45 | 46 | 43 | 40 | 47 | 48 | 45 | 45 | 44 | 45 | 41 |
| 46.0 | 41.0 | 21.5 | 34.0 | 44.0 | 34.0 | 41.0 | 38.5 | 39.5 | 50.0 | 32.5 | 50.0 | 12.0 | 34.0 | 40.0 | 50.0 | 36.0 | 41.0 | 34.0 | 35.0 | 44.0 | 30.0 |
| 41.7 | 44.0 | 46.5 | 41.0 | 45.2 | 47.3 | 42.5 | 46.9 | 43.6 | 45.6 | 44.3 | 45.2 | 46.6 | 43.5 | 40.2 | 47.8 | 48.0 | 45.2 | 45.2 | 44.5 | 45.2 | 41.6 |
| 62 | 130 | 185 | 110 | 10 | 30 | 10 | 30 | 60 | 13 | 139 | 65 | 20 | 100 | 90 | 20 | 78 | 90 | 100 | 79 | 90 | 13 |
| 28 | 41 | 24 | 36 | 38 | 38 | 38 | 34 | 40 | 26 | 34 | 41 | 26 | 44 | 26 | 40 | 30 | 32 | 36 | 28 | 50 | 36 |
| 117 | 166 | 178 | 189 | 211 | 222 | 236 | 244 | 248 | 250 | 257 | 276 | 308 | 370 | 401 | 410 | 426 | 427 | 438 | 440 | 444 | 459 |