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Research Article

Morphological spectrum of gamma rays and EMS induced viable mutants in cowpea (Vigna unguiculata (L.) Walp)

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Abstract
Seeds of cowpea (Vigna unguiculata (L.) Walp) variety CO 7 and Tirunelveli local were treated with gamma ray doses at 150, 200, 250, 300 and 350 Gy and EMS (Ethyl Methane Sulphonate) doses at 5, 10, 15, 20 and 25 mM. The total of 248 mutants from gamma irradiated population and 345 mutants from EMS treatments were identified. More number of viable mutants were recorded for EMS than gamma ray treatment in both the varieties. In variety CO 7, the total of 165 mutants were observed in the gamma ray and 251 mutants in EMS treated plants. In Tirunelveli local, the total of 83 and 94 mutants were observed for gamma ray and EMS treatment respectively. The gamma ray dose of 200 Gy and EMS dose of 10 mM recorded the highest frequency of viable morphological mutants.

Keywords
Cowpea; CO 7; Tirunelveli local; gamma ray; EMS; viable mutants

INTRODUCTION
Cowpea (Vigna unguiculata (L.) Walp) is an annual herbaceous legume from the genus Vigna. Due to its tolerance for sandy soil and low rainfall, it is an important crop in the semi-arid regions across Africa and Asia. Being a fast growing crop, cowpea curb erosion by covering the ground fixes atmospheric nitrogen and its decaying residues contribute to soil health. Cowpea is consumed in many forms: the young leaves, green pods, green seeds as vegetables and dry seeds are used in various recipes. Cowpea is a versatile kharif pulse crop because of its smothering nature, drought tolerant characters, soil restoring properties and multipurpose uses (Deepa et al., 2010).

Despite the rich germplasm collection available in various breeding programs the genetic base for the cowpea is narrow for economic traits such as grain yield, yield components, drought and insect pest tolerance (Horn and Shimelis, 2013). Various breeding methods like recombination breeding, mutation breeding and transgenic breeding, each with its unique way of generating variation and selecting target line, are utilized in the crop improvement programme. Crop Improvement of pulses through hybridization and recombination is very difficult, because of their autogamous nature. Due to their autogamous nature, lack genetic variability. The spontaneous mutation rate is pretty low and can't be exploited for breeding and that is why artificially mutations are induced with physical and chemical mutagen treatment. According to Micke et al. (1993) only eight out of 1000 improved mutant varieties of different crops released up to 1989 in over 48 countries were cowpea. It is an established fact that mutagen, besides causing changes in major genes, also induces mutations at loci governing the quantitative characters. Mutagen agents, including gamma rays, offered great possibilities for increasing genetic variability of quantitative traits such as yield. Since induced mutations are useful to produce new genetic variation and select favourable mutants, systematic study of induced mutagenesis by physical mutagens (Gamma ray) and chemical mutagens (EMS) in cowpea was attempted.

MATERIALS AND METHODS
Two cowpea varieties viz., CO 7 and Tirunelveli local were treated with Gamma ray in a ⁶⁰ Co chamber available
at Sugarcane Breeding Institute (SBI), Coimbatore. 300 dried and healthy seeds of above varieties were treated with gamma rays at five different doses from 150 to 350 Gy with an interval of 50 Gy and Ethyl Methane Sulphonate (EMS) at five levels of doses from 5 to 25 mM with an interval of 5mM. The treated seeds were sown along with control seeds of both the varieties in Randomized Block Design (RBD) at three replications with the spacing of 45 x 20 cm during Rabi season of 2014. The recommended agronomic practices and plant protection measures were followed uniformly for all the treatments. The M$_2$ generation was raised from individual M$_1$ plant following plant to the progeny method in both the varieties namely Co 7 and Tirunelveli local. A total set around 100 (75 from CO 7 and 25 from Tirunelveli Local) M$_1$ plants seeds from two varieties were forwarded to M$_2$ generation. The M$_2$ seeds of each M$_1$ plants were sown during February 2015 without replication with the spacing of 45 x 25 cm. The standard agronomic practices were followed throughout the period of crop growth as like that of M$_2$ generation. In M$_2$ generation, the occurrence of chlorophyll mutants was observed in the nursery when the seedlings were with 2-3 leaves just to assess the effect of mutagen on the biological materials. The number of M$_2$ families evaluated in CO 7 and Tirunelveli local are furnished in Table 1. The induced mutations in the plant morphology of the two varieties were categorized into six major phenotypic categories viz. plant size, growth habit, leaf, flower, pod and seed. Each category includes various mutant phenotypes related to that particular morphology and frequencies of the mutation in each morphological category out of the total morphological mutations were calculated throughout the growing season of M$_2$ generations. (Table 4 and 5).

Table 1. Number of M$_2$ families evaluated for CO 7 and Tirunelveli Local

| Variety          | Gamma rays | 150 Gy | 200 Gy | 250 Gy | 300 Gy | 350 Gy | Total |
|------------------|------------|--------|--------|--------|--------|--------|-------|
| CO 7             | 15         | 15     | 15     | 15     | 15     | 75     |
| Tirunelveli Local| 5          | 5      | 5      | 5      | 5      | 25     |
| EMS              | 5 mM       | 10 mM  | 15 mM  | 20 mM  | 25 mM  |        |
| CO 7             | 15         | 15     | 15     | 15     | 15     | 75     |
| Tirunelveli Local| 5          | 5      | 5      | 5      | 5      | 25     |

RESULT AND DISCUSSION

In the present investigation, viable macro mutations with changes in attributes like stature, duration, cotyledon, stem, leaf, pod, flower and seed mutants were recorded. Stature mutants namely dwarf, tall, and duration mutants like early and late mutants were observed in both the varieties viz., Co 7 and Tirunelveli local. (Fig.1). Plants in control did not produce any morphological mutant. As given in Table 2 and 3, the frequency of morphological abnormalities increased with increase in the dose of gamma rays till 200 Gy and 10 mM in chemical treatment followed by a decline. Most of the induced mutants were found to be fall under leaf mutations (27%) and pod mutations (22%) category followed by a duration (16%) and plant height (8%) in CO 7 variety whereas leaf mutations (21%) and stem mutations (29%) category followed by pod (12%) and duration mutations (11%) in Tirunelveli Local respectively. (Fig. 4 and 5).

Table 2. Percentage mutated plant in CO7

| Treatment | No of plants scored | Plant Height | Durations Sterile | Leaf Single Cotyledon | Stem Flower | Pod Seed Colour | Chimera Others | TMP | % Mutated plants |
|-----------|---------------------|--------------|-------------------|-----------------------|-------------|-----------------|----------------|-----|------------------|
| Gamma     |                     |              |                   |                       |             |                 |                |     |                  |
| 150Gy     | 620                 | 4            | 9                 | 0                     | 10          | 0               | 3              | 1   | 7                | 0   | 0               | 37  | 5.97            |
| 200Gy     | 602                 | 7            | 11                | 0                     | 12          | 1               | 4              | 2   | 10               | 4   | 0               | 51  | 8.47            |
| 250Gy     | 583                 | 1            | 3                 | 1                     | 10          | 0               | 4              | 1   | 10               | 5   | 0               | 34  | 5.83            |
| 300Gy     | 446                 | 2            | 3                 | 0                     | 9           | 0               | 2              | 1   | 6                | 2   | 0               | 25  | 5.61            |
| 350Gy     | 248                 | 1            | 2                 | 0                     | 5           | 0               | 3              | 0   | 5                | 2   | 0               | 18  | 7.26            |
| EMS       |                     |              |                   |                       |             |                 |                |     |                  |
| 5mM       | 553                 | 6            | 10                | 0                     | 12          | 0               | 4              | 2   | 14               | 4   | 0               | 52  | 9.40            |
| 10mM      | 550                 | 8            | 13                | 0                     | 14          | 2               | 8              | 4   | 18               | 4   | 1               | 72  | 13.09           |
| 15mM      | 536                 | 4            | 5                 | 2                     | 16          | 0               | 8              | 2   | 7                | 4   | 0               | 48  | 8.96            |
| 20mM      | 502                 | 2            | 6                 | 0                     | 13          | 0               | 8              | 3   | 8                | 3   | 0               | 43  | 8.57            |
| 25mM      | 476                 | 0            | 5                 | 0                     | 12          | 0               | 10             | 0   | 5                | 2   | 0               | 34  | 7.14            |

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Fig. 1. Different types of morphological mutants observed in CO 7 and Tirunelveli Local in M2 generation

A. Tall and Dwarf

B. Flower color

C. Stem pigmentation

D. Pod Variation

E. Sterile plants

F. Chimeras

G. Wavy leaf margins

H. Seed coat colour
### Table 3. Percentage mutated plant in Tirunelveli Local

| Treatment | No of plants scored | Morphological mutant types of Tirunelveli Local |  |  |  | TMP | % Mutated plants |
|-----------|---------------------|-----------------------------------------------|---|---|---|----|-----------------|
| **Gamma** |                     |                                               |   |   |   |    |                 |
| 150Gy     | 425                 | Plant Height: Tall 1, Dwarf 2, Erect 3         | 1 | 2 | 1 | 3 | 0 | 5 | 1 | 3 | 2 | 0 | 0 | 18 | 4.24 |
| 200Gy     | 418                 | Duration: Sterile 4, Leaf Single cotyledon 0, Stem Flower 7, Pod 0, Seed Colour 3, Chimera 1, Others 0 | 0 | 1 | 1 | 3 | 0 | 5 | 0 | 0 | 2 | 1 | 0 | 13 | 3.92 |
| 250Gy     | 350                 |ök | 0 | 1 | 2 | 0 | 5 | 0 | 0 | 2 | 1 | 0 | 11 | 3.67 |
| 300Gy     | 332                 | 2 | 0 | 1 | 2 | 0 | 5 | 0 | 0 | 2 | 1 | 0 | 11 | 3.67 |
| 350Gy     | 300                 | 2 | 0 | 1 | 2 | 0 | 5 | 0 | 0 | 2 | 1 | 0 | 11 | 3.67 |
| **EMS**   |                     |                                               |   |   |   |    |                 |
| 5mM       | 410                 | 3 | 2 | 2 | 4 | 0 | 7 | 1 | 2 | 1 | 0 | 0 | 22 | 5.37 |
| 10mM      | 402                 | 2 | 3 | 0 | 6 | 1 | 10 | 1 | 5 | 2 | 0 | 0 | 34 | 8.46 |
| 15mM      | 343                 | 0 | 0 | 1 | 2 | 0 | 4 | 0 | 2 | 1 | 0 | 0 | 15 | 4.37 |
| 20mM      | 321                 | 0 | 2 | 1 | 2 | 0 | 4 | 0 | 3 | 1 | 0 | 0 | 13 | 4.05 |
| 25mM      | 305                 | 0 | 1 | 0 | 5 | 0 | 3 | 0 | 2 | 1 | 0 | 0 | 12 | 3.93 |

### Table 4. Frequency and spectrum of morphological mutants induced by various mutagens in M₂ generation of CO7

| TYPE OF MUTANT | MUTAGEN DOSE OF CO7 |
|---------------|---------------------|
|               | GAMMA RAYS (Gy)    | EMS | Total | Grand Total |
|               | N | F% | N | F% | N | F% | N | F% |
| **Plant Height** |   |     |   |     |   |    |   |    |
| Tall          | 4 | 0.16 | 6 | 0.23 | 10 | 0.20 | 35 | 0.68 |
| Dwarf         | 8 | 0.32 | 8 | 0.31 | 16 | 0.31 | 34 | 0.62 |
| Erect         | 3 | 0.12 | 6 | 0.23 | 9 | 0.18 | 15 | 0.26 |
| **Duration mutants** |   |     |   |     |   |    |   |    |
| Early mutant  | 21 | 0.84 | 27 | 1.03 | 48 | 0.94 | 67 | 1.31 |
| Late mutant   | 7 | 0.28 | 12 | 0.46 | 19 | 0.37 | 36 | 0.65 |
| **Sterile mutants** |   |     |   |     |   |    |   |    |
| Sterile       | 1 | 0.04 | 2 | 0.08 | 3 | 0.06 | 3 | 0.06 |
| **Cotyledonal abnormalities** |   |     |   |     |   |    |   |    |
| Single cotyledon | 1 | 0.04 | 2 | 0.08 | 3 | 0.06 | 3 | 0.06 |
| Variation in leaflet number | 35 | 1.40 | 47 | 1.80 | 82 | 1.60 | 113 | 2.21 |
| **Leaf modification** |   |     |   |     |   |    |   |    |
| Other leaf mutants | 10 | 0.40 | 20 | 0.76 | 30 | 0.59 | 113 | 2.21 |
| Narrow        | 1 | 0.04 | 0 | 0.00 | 1 | 0.02 | 1 | 0.02 |
| Spares        | 7 | 0.28 | 10 | 0.38 | 17 | 0.33 | 52 | 1.02 |
| **Stem Pigmentation** |   |     |   |     |   |    |   |    |
| Entire        | 7 | 0.28 | 28 | 1.07 | 35 | 0.68 | 52 | 1.02 |
| **Flower modification** |   |     |   |     |   |    |   |    |
| White with blue stripes | 2 | 0.08 | 3 | 0.11 | 5 | 0.10 | 16 | 0.31 |
| Light blue colour | 3 | 0.12 | 8 | 0.31 | 11 | 0.22 | 17 | 0.30 |
| Small pods    | 8 | 0.32 | 7 | 0.27 | 15 | 0.29 | 21 | 0.41 |
| Long pods     | 5 | 0.20 | 14 | 0.53 | 19 | 0.37 | 29 | 0.55 |
| **Pod modification** |   |     |   |     |   |    |   |    |
| Colour variation | 1 | 0.04 | 4 | 0.14 | 8 | 0.16 | 14 | 0.27 |
| **Changes in seed character** |   |     |   |     |   |    |   |    |
| Constriction  | 24 | 0.96 | 30 | 1.15 | 54 | 1.06 | 80 | 1.57 |
| Bold seed     | 2 | 0.08 | 0 | 0.00 | 2 | 0.04 | 2 | 0.04 |
| Seed coat colour | 15 | 0.60 | 17 | 0.65 | 32 | 0.63 | 48 | 0.91 |
| Others        | 0 | 0.00 | 1 | 0.04 | 1 | 0.02 | 1 | 0.02 |
| Other         | 0 | 0.00 | 2 | 0.08 | 2 | 0.04 | 2 | 0.04 |
| **Total**     | 165 | 6.60 | 251 | 9.59 | 416 | 8.13 | 416 | 8.13 |
Table 5. Frequency and spectrum of morphological mutants induced by various mutagens in M$_2$ generation of Tirunelveli local

| TYPE OF MUTANT       | MUTAGEN DOSE OF TIRUNELVELI LOCAL | GAMMA RAYS (Gy) | EMS | Total | Grand Total |
|----------------------|-----------------------------------|----------------|-----|-------|-------------|
| Plant Height         |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| Tall                 | 2                                 | 0.11 | 5  | 0.28 | 7  | 0.19 | 12  | 0.33 |
| Dwarf                | 0                                 | 0.00 | 2  | 0.11 | 2  | 0.06 | 12  | 0.33 |
| Erect                | 1                                 | 0.05 | 2  | 0.11 | 3  | 0.08 | 6   | 0.17 |
| Duration mutants     |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| Early mutant         | 6                                 | 0.33 | 7  | 0.39 | 13 | 0.36 | 19  | 0.53 |
| Late mutant          | 3                                 | 0.16 | 3  | 0.17 | 6  | 0.17 | 8   | 0.22 |
| Sterile mutants      |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| Sterile              | 4                                 | 0.22 | 4  | 0.22 | 8  | 0.22 | 8   | 0.22 |
| Cotyledonary         |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| abnormalities        | Single cotyledon                  | 1   | 0.05 | 1   | 0.06 | 2   | 0.06 | 2   | 0.06 |
|                      | Variation in leaflet number       | 14  | 0.77 | 18  | 1.01 | 32  | 0.89 | 37  | 1.03 |
| Leaf modification     |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| Other leaf mutants   | 2                                 | 0.11 | 3  | 0.17 | 5  | 0.14 | 37  | 1.03 |
| Narrow               | 0                                 | 0.00 | 0  | 0.00 | 0  | 0.00 | 0   | 0.00 |
| Spares               | 10                                | 0.55 | 13 | 0.73 | 23 | 0.64 | 53  | 1.47 |
| Entire               | 13                                | 0.71 | 17 | 0.95 | 30 | 0.83 | 53  | 1.47 |
| Stem Pigmentation    |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| White with blue stripes | 1                              | 0.05 | 0  | 0.00 | 1  | 0.03 | 7   | 0.19 |
| Light blue colour    | 4                                 | 0.22 | 2  | 0.11 | 6  | 0.17 | 21  | 0.58 |
| Small pods           | 0                                 | 0.00 | 0  | 0.00 | 0  | 0.00 | 0   | 0.00 |
| Long pods            | 3                                 | 0.16 | 4  | 0.22 | 7  | 0.19 | 21  | 0.58 |
| Pod modification      |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| Colour variation     | 3                                 | 0.16 | 0  | 0.00 | 3  | 0.08 | 17  | 0.47 |
| Constriction         | 4                                 | 0.22 | 7  | 0.39 | 11 | 0.31 | 17  | 0.47 |
| Changes in seed      |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| character            | Bold seed                         | 0   | 0.00 | 0  | 0.00 | 0  | 0.00 | 0   | 0.00 |
| Seed coat colour     | 11                                | 0.60 | 6  | 0.34 | 17 | 0.47 | 17  | 0.47 |
| Others               |                                   | N   | F%  | N   | F%  | N   | F%  | N   | F%  |
| Chimeric mutant      | 1                                 | 0.05 | 0  | 0.00 | 1  | 0.03 | 1   | 0.03 |
| Total                |                                   | 83  | 4.55 | 94  | 5.28 | 177 | 4.91 | 177 | 4.91 |

Fig.2. The comparative estimation of phenotypic mutants induced by Gamma radiation and EMS in CO7
Fig. 3. The comparative estimation of phenotypic mutants induced by Gamma radiation and EMS in Tirunelveli Local

Fig. 4. Spectrum of viable mutants in Tirunelveli local
In the present study, different frequencies of occurrence were observed not only between the two varieties but also within the mutagenic treatments thereby signifying the mutagen type and concentration dependency for inducing macromutations. Based on morphological mutation frequency, the variety Tirunelveli Local was found to be comparatively less mutable than the variety CO7. (Fig.2 and 3)

Tall mutants were characterized by long internodes. These mutants appeared more frequently in EMS treatments. Tall mutants, as observed in the present study, were also reported earlier by Solanki et al. (2005) in lentil, Kumar et al. (2009) in black gram and Goyal et al. (2019) in black gram. Early and late mutants have been reported by Dhanavel et al. (2012) in cowpea; Rudraswami et al. (2006) and Dhumal and Bolbhat (2012) in horse gram. Sterile mutants were found to be induced by both gamma rays and EMS treatments. A similar type of mutants has been reported by Adekola and Oluleye, (2007) for gamma ray induced mutation in cowpea. Seedlings with single cotyledon were reported by Banu (2000) for gamma rays and EMS treatments. Similar with mutants reported by Banu (2000) and Mishra and Chand, (2003) in cowpea. The mutants exhibited brownish white seed coat colour in Tirunelveli local was also reported by Singh and Yadav (1991) in greengram and Ashok et al. (2010). Chimeric mutants were identified in gamma ray treatments in Tirunelveli local and in EMS treatments in CO7 which consonances with Thakur (2004) and Gnanamurthy et al. (2012) in cowpea. Bhat et al. (2006) in Vicia faba (L.).

The variety Co7 produced 165 and 251 viable mutants in gamma rays and EMS treatment respectively. In Tirunelveli local, gamma rays and EMS treatment produced 83 and 94 viable mutants respectively. Total morphological mutation frequency was more prominent in EMS (9.59%) (5.28%) than gamma radiation (6.60 %) (4.55%) in CO7 and Tirunelveli local respectively. This finding revealed the better efficiency of alkylating agents in inducing point mutations than irradiation. The frequency of viable mutants was higher in EMS treatments on M2 plant basis was corroborated with Nair and A.K. Mehta (2014) in cowpea, Dhumal and Bolbhat (2012) in Horsegram and
Ramesh et al (2019) in barnyard millet whereas, this was in contrast to the findings by (Senapati et al., 2008; Khursheed et al 2017) and Ramchander et al (2017) stating gamma radiation the efficient one than EMS.

Among the ten doses of treatment in gamma rays and EMS, the dose 200 Gy and 10 mM Mj registered the highest frequency of viable mutants in M2 generation in both the varieties. (Table 2 and 3).

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