



Induction and characterisation of agronomically useful mutants in horsegram (*Macrotyloma uniflorum*)

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Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] (2n=20) is a lesser known but an important pulse crop, being cultivated in the diverse climatic conditions of the world. It is considered to be the most tolerant to biotic and abiotic stresses (Jeswani and Baldev 1990). Besides, it has many potential nutraceutical uses and its lipids are known to be protective and promote healing effects on acute gastric ulceration produced by excessive alcohol consumption (Jayraj *et al.* 2000). It is one of the major constituents of Cystone syrup used to dissolve and dislocate kidney stones in humans (Jeyaraman *et al.* 2007). Despite the presence of many significant properties in the species, the area and production could not be increased due to its poor plant architecture. The present plant type possesses many wild characteristics such as indeterminate and twining growth habit, photosensitivity, late flowering and asynchronous maturity, resulted into unsuitability for modern farming system. All the efforts in the past to improve the existing plant structure of this crop proved futile due to non-availability of desirable traits in the

Indian germplasm. The present investigation therefore, was carried out with the objectives to induce desirable mutations in the well adapted promising lines of horsegram and further to identify suitable genotypes for cultivation in different agro-climatic conditions of the country.

Two promising lines HPKC 2 and VLG 1 having thin twining type long stem, indeterminate growth habit, photosensitivity, asynchronous and late maturity were selected on the basis of multiyear evaluation data at the experimental farm of the Department of Crop Improvement, CSK Himachal Pradesh Agricultural University, Palampur. Different parameters were used to calculate LD₅₀ dose. The slightly lower dose to LD₅₀ (150Gy), close to LD₅₀ (250Gy) and slightly higher dose to LD₅₀ (350Gy) were chosen to irradiate both the varieties (Table 1).

Three thousand seeds each of two varieties were irradiated with 150, 250, and 350 Gy of gamma rays. The M₁ generation of the irradiated seeds were space planted and at maturity, seeds from each plant were harvested separately to grow the M₂ generation. Plant to progeny rows were raised in an Augmented Block Design along with four checks. Data were recorded on 22 600 plants for identifying morphological

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Table 1 Effectiveness of different doses of gamma radiations for induction of desirable traits in HPKC 2 and VLG 1

| Trait | Dose | | | Total | Dose | | | Total | Total mutants in both the varieties | % age of desirable mutations |
|--------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------------------------------------|------------------------------|
| | 150Gy | 250Gy | 350Gy | | 150Gy | 250Gy | 350Gy | | | |
| | HPKC 2 | | | | VLG 1 | | | | | |
| Early flowering | 0 | 7 | 0 | 7 | 1 | 0 | 0 | 1 | 8 | 0.035 |
| Early maturing | 1 | 6 | 0 | 7 | 1 | 0 | 0 | 1 | 8 | 0.035 |
| Semi-dwarf | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 1 | 3 | 0.013 |
| Dwarf | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0.022 |
| Bushy growth habit | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 5 | 0.022 |
| Determinate Growth habit | 1 | 6 | 0 | 7 | 1 | 0 | 0 | 1 | 8 | 0.035 |
| Photo insensitive | 1 | 6 | 0 | 7 | 1 | 0 | 0 | 1 | 8 | 0.035 |
| Chlorina type | 0 | 6 | 3 | 9 | 0 | 5 | 2 | 7 | 16 | 0.07 |
| Chlorophyll mutants | | | | | | | | | | |

Table 2 Effect of the mutagen (Gamma radiation) on initial growth of the plant after 15 days of sowing

| Varieties | LD ₅₀ (Gy) | Root length (cm) | Shoot length (cm) | Number of leaves |
|-----------|--------------------------|---------------------|----------------------|---------------------|
| HPKC 2 | 150 | 8.42 | 0.98 | 1.02 |
| | 250 | 12.42 | 1.80 | 2.60 |
| | 350 | 12.60 | 2.86 | 3.45 |
| VLG 1 | 150 | 7.02 | 1.05 | 1.30 |
| | 250 | 14.41 | 2.80 | 2.81 |
| | 350 | 16.75 | 3.81 | 4.70 |

variations for agronomic traits. In M₂ generation, eleven morphologically distinct and agronomically superior mutants were selected and evaluated in the M₃ and M₄ generations along with two parents for 2 years in a randomised block design with three replications. Data were recorded on 10 individual plants for different agronomic and morpho-physiological traits and analysis was carried out by using standard statistical procedures.

Leaf area was measured by using leaf area meter software developed by Disha Infoway India (P) Ltd. Nodules in the mutants and parents were counted by uprooting the plants carefully from long polytubes at the time of flowering. Photosensitive reaction of selected mutants was determined by planting these mutants under three different photoperiodic regimes. One set of mutants was raised under natural conditions having photoperiod around 12 hours at the time of flowering, whereas other two sets were planted under artificial conditions and the photoperiod was kept at 8 and 16 hours lights throughout the growing season. Data for other agronomic traits such as number of primary branches/plant, number of pods/cluster, number of pods/plant, number of

seeds/pods, seed yield/plant and 100-seed weight were recorded by following the standard procedures. Data recorded in M₃ and M₄ generations for different qualitative and quantitative traits were pooled together as these mutants were not showing any variation within line for these traits.

Generally, the ionizing radiations produce two types of effect on the hereditary material: gene mutation and chromosome break. Most of the times, the frequency of these induced mutations is deleterious in nature and have not any practical significance in plant breeding, but in the present investigation, two well adapted line namely HPKC 2 and VLG 1 irradiated with gamma radiation were resulted into number of beneficial morphological changes.

Plant height has been considered as an important component determining the ideotype of any crop plant. The current plant type of most of the cultivars under cultivation is not suitable for commercial cultivation due to its thin, long and twining type growth habit. The 250 Gy dose of gamma radiation induced dwarfing genes in HPKC 2, whereas 150 Gy doses of gamma radiation induced dwarfing genes in VLG 1. Two types of dwarf mutants appeared in M₂ population. The first group consisting of 3 mutants namely, M 191, M 193 and M 249 was semi-dwarf while second group of mutants consisting of M 317, M 319, M 320, M 321 and M 322 was dwarf as compared to parents HPKC 2 and VLG 1. The percentage of semi-dwarf and dwarf mutants was 0.055% and 0.01% in HPKC 2 and VLG 1, respectively (Table 3).

Six novel photoinensitive mutants were recovered in HPKC 2 at 250Gy and one at 150Gy doses of gamma radiation. These mutants took less than 40 days to flower, whereas HPKC 2 flower in 75 days. One early flowering mutant also appeared in the variety VLG 1 at 150Gy dose of

Table 3 Performance of M₃ and M₄ mutants over years for various morpho-physiological traits

| | Morpho-physiological traits | | | | | | | | | | | | | | |
|--------|-----------------------------|------|------|------------------------------|-------|-------|-----------------------|-------|-------|---------------------|-------|-------|-------------------------|-------|-------|
| | Leaf area index | | | Leaf area (cm ²) | | | Branch angle (degree) | | | Leaf angle (degree) | | | Number of Nodules/plant | | |
| | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean |
| M 191 | 2.70 | 2.66 | 2.68 | 6.85 | 7.07 | 6.96 | 30.52 | 33.72 | 32.12 | 41.06 | 46.06 | 43.66 | 18.87 | 23.87 | 21.37 |
| M 193 | 3.32 | 3.31 | 3.32 | 8.30 | 8.36 | 8.33 | 33.35 | 37.55 | 35.45 | 48.16 | 59.28 | 53.72 | 22.38 | 28.35 | 25.36 |
| M 249 | 3.13 | 3.11 | 3.12 | 7.41 | 7.47 | 7.44 | 23.84 | 27.34 | 25.84 | 47.05 | 53.77 | 50.41 | 18.18 | 24.9 | 21.54 |
| M 317 | 2.39 | 2.33 | 2.36 | 12.15 | 12.27 | 12.21 | 24.74 | 31.74 | 28.24 | 25.58 | 30.08 | 27.83 | 27.79 | 33.39 | 30.59 |
| M 319 | 3.18 | 3.08 | 3.13 | 10.59 | 10.63 | 10.61 | 31.70 | 32.70 | 33.76 | 30.31 | 37.17 | 33.76 | 32.49 | 29.05 | 30.95 |
| M 320 | 2.44 | 2.42 | 2.43 | 10.61 | 10.77 | 10.69 | 34.12 | 38.54 | 36.34 | 32.09 | 40.59 | 36.34 | 27.05 | 24.05 | 25.55 |
| M 321 | 2.65 | 2.77 | 2.71 | 11.72 | 11.84 | 11.78 | 30.29 | 33.29 | 31.79 | 28.99 | 34.59 | 31.79 | 34.34 | 30.34 | 32.34 |
| M 322 | 3.20 | 3.30 | 3.25 | 11.27 | 11.29 | 11.28 | 26.97 | 35.97 | 31.47 | 28.97 | 33.57 | 31.47 | 42.29 | 34.29 | 38.29 |
| M 150 | 3.12 | 3.20 | 3.16 | 9.10 | 9.20 | 9.15 | 50.60 | 58.4 | 54.10 | 50.40 | 57.80 | 54.10 | 38.21 | 44.21 | 41.21 |
| M 151 | 3.10 | 3.14 | 3.12 | 9.06 | 9.12 | 9.09 | 51.36 | 59.56 | 55.46 | 51.86 | 59.06 | 55.46 | 39.02 | 46.02 | 42.52 |
| M 201 | 3.05 | 3.11 | 3.08 | 9.09 | 9.13 | 9.11 | 53.18 | 59.78 | 56.48 | 52.98 | 59.98 | 56.48 | 47.55 | 39.15 | 43.35 |
| HPKC2 | 3.19 | 3.21 | 3.30 | 8.84 | 8.94 | 8.85 | 52.75 | 59.75 | 56.25 | 54.25 | 58.25 | 56.25 | 45.84 | 39.64 | 42.74 |
| VLG 1 | 3.39 | 3.33 | 3.35 | 9.07 | 9.19 | 9.13 | 49.83 | 58.83 | 54.33 | 52.23 | 56.43 | 54.33 | 37.69 | 43.69 | 40.69 |
| SE±(m) | | | 0.23 | | | 0.46 | | | 2.33 | | | 5.89 | | | 5.56 |

Table 4 Performance of M₃ and M₄ mutants over years for various structural and phenological traits

| | Structural and phenological traits | | | | | | | | | | | |
|---------|------------------------------------|-------|-------|----------------------|--------|--------|-------------------|--------|--------|-----------------------|-------|-------|
| | Days to 50% flowering | | | Days to 75% maturity | | | Plant height (cm) | | | First pod height (cm) | | |
| | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean |
| M 191 | 40.72 | 43.52 | 42.12 | 81.05 | 88.85 | 84.55 | 65.8 | 68.20 | 67.00 | 8.66 | 6.46 | 7.56 |
| M 193 | 41.10 | 43.10 | 42.10 | 80.04 | 87.84 | 84.34 | 73.57 | 71.13 | 72.33 | 7.96 | 9.76 | 8.85 |
| M 249 | 34.38 | 37.38 | 35.88 | 79.76 | 85.86 | 82.26 | 55.03 | 57.63 | 56.33 | 10.14 | 8.54 | 9.34 |
| M 317 | 38.74 | 41.94 | 40.34 | 95.60 | 104.6 | 100.26 | 31.03 | 33.63 | 32.33 | 5.35 | 6.55 | 5.95 |
| M 319 | 41.53 | 45.13 | 43.33 | 97.30 | 105.10 | 101.20 | 32.03 | 34.63 | 33.33 | 5.75 | 7.35 | 6.55 |
| M 320 | 41.17 | 44.17 | 42.67 | 100.96 | 105.96 | 102.76 | 29.30 | 31.70 | 30.50 | 6.54 | 7.94 | 7.24 |
| M 321 | 41.75 | 46.15 | 43.95 | 103.12 | 106.12 | 104.62 | 31.90 | 35.10 | 33.50 | 7.35 | 8.65 | 8.00 |
| M 322 | 39.83 | 44.53 | 42.33 | 101.00 | 104.60 | 102.80 | 32.83 | 29.83 | 31.33 | 5.72 | 6.82 | 6.32 |
| M 150 | 62.74 | 68.34 | 65.54 | 130.25 | 135.25 | 132.75 | 103.1 | 105.65 | 104.35 | 12.95 | 15.95 | 14.45 |
| M 151 | 63.65 | 69.65 | 66.65 | 125.68 | 132.68 | 131.18 | 97.45 | 101.45 | 99.45 | 14.50 | 14.50 | 15.75 |
| M 201 | 66.74 | 69.94 | 68.34 | 129.35 | 131.55 | 130.45 | 98.90 | 101.10 | 100.25 | 13.92 | 16.85 | 15.22 |
| HPKC2 | 58.17 | 65.17 | 61.67 | 134.45 | 137.25 | 135.85 | 101.63 | 104.03 | 102.83 | 15.24 | 17.46 | 16.35 |
| VLG 1 | 71.58 | 79.38 | 75.48 | 130.84 | 134.64 | 132.74 | 94.66 | 100.66 | 99.36 | 13.89 | 16.69 | 15.29 |
| SE+ (m) | | | 7.35 | | | 11.46 | | | 14.28 | | | 3.82 |

Table 5 Performance of M₃ and M₄ mutants over years for various seed yield traits

| | Seed yield traits | | | | | | | | | | | | | | |
|---------|-------------------|--------|--------|-----------|------|------|--------------|------|------|---------------------|------|------|-------------------|-------|-------|
| | Pods/plant | | | Seeds/pod | | | Pods/cluster | | | 100-seed weight (g) | | | Grain yield/plant | | |
| | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean | 2008 | 2009 | Mean |
| M 191 | 86.95 | 79.95 | 83.45 | 5.76 | 6.16 | 5.96 | 4.19 | 3.69 | 3.94 | 3.21 | 3.23 | 3.22 | 9.21 | 7.71 | 8.46 |
| M 193 | 96.63 | 88.63 | 92.63 | 5.12 | 5.72 | 5.42 | 5.50 | 6.20 | 5.25 | 3.12 | 3.18 | 3.15 | 9.76 | 8.70 | 9.24 |
| M 249 | 90.11 | 96.77 | 93.44 | 6.06 | 5.62 | 5.84 | 4.66 | 4.44 | 4.55 | 3.00 | 3.12 | 3.06 | 7.85 | 8.42 | 8.17 |
| M 317 | 133.08 | 143.08 | 138.58 | 6.47 | 4.97 | 5.72 | 6.28 | 4.92 | 5.25 | 3.24 | 3.23 | 3.23 | 9.51 | 10.42 | 9.96 |
| M 319 | 76.07 | 83.07 | 79.57 | 5.77 | 6.47 | 6.12 | 5.59 | 6.31 | 5.95 | 3.05 | 3.13 | 3.09 | 11.23 | 8.77 | 10.00 |
| M 320 | 59.89 | 68.89 | 64.39 | 5.78 | 6.22 | 6.00 | 5.69 | 5.09 | 5.34 | 3.03 | 2.97 | 3.00 | 8.54 | 7.80 | 8.16 |
| M 321 | 80.92 | 88.52 | 84.72 | 6.11 | 5.39 | 5.75 | 5.89 | 5.45 | 5.67 | 3.12 | 3.14 | 3.13 | 10.51 | 10.63 | 11.07 |
| M 322 | 107.95 | 108.45 | 103.95 | 5.70 | 6.30 | 6.00 | 4.80 | 5.20 | 4.00 | 3.29 | 3.33 | 3.31 | 11.80 | 15.50 | 13.65 |
| M 150 | 87.75 | 97.55 | 92.65 | 4.68 | 4.22 | 4.45 | 2.15 | 2.45 | 2.30 | 3.29 | 3.23 | 3.26 | 8.68 | 11.78 | 10.23 |
| M 151 | 98.83 | 89.83 | 94.33 | 7.07 | 5.79 | 6.24 | 2.43 | 2.57 | 2.50 | 3.35 | 3.33 | 3.34 | 13.33 | 10.00 | 11.67 |
| M 201 | 79.39 | 91.39 | 85.39 | 6.09 | 7.15 | 6.62 | 3.05 | 3.25 | 3.15 | 3.14 | 3.10 | 3.12 | 10.60 | 9.10 | 9.85 |
| HPKC2 | 89.85 | 80.85 | 85.35 | 6.61 | 5.91 | 6.26 | 2.62 | 3.14 | 2.88 | 3.16 | 3.12 | 3.14 | 13.13 | 11.37 | 12.25 |
| VLG 1 | 87.08 | 75.96 | 81.52 | 6.23 | 5.16 | 5.67 | 2.63 | 2.33 | 2.48 | 3.59 | 3.59 | 3.59 | 14.29 | 12.39 | 13.39 |
| SE+ (m) | | | 10.56 | | | 0.78 | | | 0.69 | | | 0.11 | | | 1.39 |

gamma radiation.

The indeterminate growth habit accompanied by asynchronous maturity is a major bottleneck to promote this crop for commercial cultivation. The parental lines HPKC 2 and VLG 1 were having indeterminate growth habit as flowering progressed from the base to tip of the plant with continuous growth of the apical and auxiliary buds. Eight mutants with determinate growth habit and synchronous maturity were obtained at 250 Gy and 150 Gy dose of gamma radiation. Five of the 8 mutants namely; M 317, M 318, M 319, M 320 and M 321 were bushy in appearance

accompanied by determinate growth habit and medium maturity. Three mutants namely, M 191, M 193 and M 249 were determinate in growth habit and very early maturing, however owing to increased plant height their appearance was not busy. The narrow leaf angle between the branch and main stem provides the mutants additional advantage of improved photosynthetic efficiency.

Higher number of pods per cluster is another important trait influencing the seed yield of pulse crops. The new plant type was having 3.94-5.85 pods/cluster which were placed centrally on the plant. The decrease in plant height has not

effected the yield potential of the mutants as this possessed approximately same number of pods/plant as compared to the parental lines.

The average number of primary branches/plant is an important traits while selecting plant for improved architecture. The semi-dwarf group of mutants namely, M 191, M 193 and M 249 had less number of primary branches/plant ranging from 2.62 to 3.00 (Table 3), whereas the dwarf mutants M 317, M 318, M 320, M 321 and M 322 had 6.26-7.73 primary branches/plant. The leaf area of three semi-dwarf mutants was smaller than that of parental lines, whereas five dwarf mutants had larger leaf area than the parental lines. All the mutants have the same seed size as of parents; however mutants M 151 and M 201 showed dark brown and brown seed colour while M 150 was white coloured.

The semi dwarf group of mutants named as Ideotype-I (Fig 1) having medium plant height, very early flowering and maturity, small leaf size with yellowish green colour. This new plant type took 35-42 days to flower and 82-84 days to mature, which was about 45 days earlier than the parents. Therefore, owing to shorter growing period, these mutants can be grown as a catch crop between two main crops. Another group of mutants designated as Ideotype-II as shown in Fig 1, consisting of M 317, M 319, M 320, M 321 and M 322 was dwarf in height possessing determinate growth habit, 4-5 pods/cluster, photo insensitivity and early flowering accompanied by synchronous and medium maturity.

In literature there are numerous examples in other crops where development of determinate and photo insensitive plant type made them commercial viable. Development of "Bush Blue lake" determinate snap beans from traditional pole type cultivars helped it to retain a vital industry in the Pacific Northwest (Evans 1980) and development of dwarf genotypes in soybean enabled it to become a commercially viable crop (Byth and Weber 1968). In the past various workers have attempted to induce desirable mutation using gamma radiations in horsegram (Gupta and Sharma 1996, Jamadagni *et al.* 1996, Shirsat *et al.* 2010, Reddy *et al.* 2010).

The appearance of desirable mutants in present investigation confirmed that it is possible to induce and select determinate and photoinsensitive mutants with synchronous maturity by using the gamma radiation as a mutagen. The selection of unique mutants having many desirable traits over their parents was not a matter of chance; rather, it required the use of several parent cultivars for mutagenesis, a reasonable population size, a careful screening



Fig 1 The new Ideotypes of horsegram

system and mutagenic efficiency and effectiveness in order to avoid drastic changes as the direct use of the mutant was of primary concern (Cagirgan 2006). Evaluation of newly induced mutants for two years revealed that 8 mutants were photoinsensitive and possessing most of the desirable traits that would allow their cultivation in different agro-climatic conditions of India. In addition these mutants can serve as a valuable source of germplasm in future horsegram breeding programme.

SUMMARY

Two well adapted horsegram lines namely, HPKC 2 and VLG 1 were irradiated with gamma rays to create novel variation and to modify existing plant type. Three doses of gamma radiation, viz. 150, 250 and 350 Gy were used to irradiate 1 000 seeds each. The M₂ generation exhibited three types of mutations which were morphologically different from the parental lines for one or the other traits. The first group of mutants namely, M 191, M 193 and M 249 were semi-dwarf, determinate, photoinsensitive, very early flowering with early and synchronous maturity. The second group of mutants, viz. M 317, M 318, M 319, M 321 and M 322 were dwarf, determinate accompanied by bushy growth habit, photo insensitive and synchronous medium maturity and third group of mutants, viz. M 150, M 151 and M 201 only differed for seed colour. All these mutants were of immense practical significance as these can be used directly as a new variety with improved agronomic traits or can be a novel source for these traits in future breeding programmes.

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