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An induced brachytic mutant of chickpea and its possible use in ideotype breeding

P. M. Gaur · V. K. Gour · S. Srinivasan

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Abstract Mutations were induced in chickpea (*Cicer arietinum* L.) cultivar 'JG 315' through treatment of seeds with ethyl methane sulphonate (EMS). One of the mutants, named JGM 1, had brachytic growth (compact growth), characterized by erect growth habit, thick and sturdy stem, short internodal and interleaflet distances and few tertiary and later order branches. It was isolated from M_2 derived from seeds treated with 0.6% EMS for 6 h. Segregation analyses in F_2 progenies of its crosses with normal chickpea genotypes (JG 315, ICC 4929, and ICC 10301) suggested that a single recessive gene controlled brachytic growth in JGM 1. This gene was not allelic to the *br* gene for brachytic growth in spontaneous brachytic mutant E100YM. Thus, the gene for brachytic growth in JGM 1 was designated *br2* and the *br* gene of E100YM was redesignated *br1*. Efforts are being made to use JGM 1 in development of a plant type with short internodes and erect growth habit. Such plant type may resist excessive vegetative growth in high input (irrigation and fertility) conditions and accommodate more plants per unit area.

Keywords *Cicer arietinum* · Brachytic · Compact growth · Ideotype breeding · Inheritance · Induced mutation · Short internodes

P. M. Gaur (✉) · S. Srinivasan
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324 AP, India
e-mail: p.gaur@cgiar.org

V. K. Gour
Department of Plant Breeding and Genetics, Jawaharlal Nehru Agricultural University, Jabalpur 482 004 MP, India

Introduction

Chickpea (*Cicer arietinum* L.) is the second largest grown food legume of the world covering an area of 11.2 million ha across 49 countries (FAOSTAT data 2006). About 97% of the chickpea area is in developing countries, where it is largely grown under marginal conditions of moisture stress and low fertility. One of the reasons for not growing chickpea in high input conditions is that the available varieties do not respond favorably to high fertility and irrigated conditions. As chickpea has indeterminate growth habit, excess water promotes vegetative growth, which acts as competitive sink for developing pods and seeds (Khanna-Chopra and Sinha 1990). Although one or two irrigations considerably enhance the yield, particularly in moisture stress conditions, a decline in yield occurs at higher irrigation levels due to excessive vegetative growth of the plants and poor pod set (Sinha et al. 1985). On the other hands in cereals, the amount of water available after anthesis has a linear relationship with yield (Passioura 1976). Thus, when farmers have fertile soils and assured sources to support higher number of irrigations, they prefer to grow a crop, such as wheat, that respond well to high input conditions. This is evident from the progressive shift of chickpea area from the traditional high productive regions of northern India toward dry regions in central and peninsular India (Lal 1992).

Restructuring of plant type, which was a key to enhancing productivity of wheat and rice, is needed for bringing a breakthrough in chickpea productivity. Attempts have been made to define ideotype of chickpea for different growing conditions. Jain (1975) suggested that major gains in chickpea yield could be achieved by developing a plant type with high harvest index, response to increased plant population per unit area, and early maturity. He further suggested that improvement in harvest index would likely to be associated with determinate and compact growth habit. Bahl and Jain (1977) included erect growth habit, many primary and secondary branches and few tertiary and later order branches in chickpea ideotype. According to them this plant type would intercept more sunlight and permit large population per unit area. Sedgley et al. (1990) also emphasized that an ideotype for high input environments should have erect growth habit and limited branching.

Efforts have been made to breed for erectness combined with tall growth by hybridization between conventional spreading and tall types (Bahl 1980). However, no significant improvement in harvest index was achieved because the internodal length of the resultant progenies was large and substantial assimilates were spent on structural parts of the plant (Khanna-Chopra and Sinha 1987). An emphasis on short internodes and compact growth in the ideotype has been given by several researchers (Ramanujam 1975; Sinha 1977; Gupta and Lal 1981; Dahiya and Lather 1990). Such plant type is expected to give high harvest index accommodate more plants per unit area and resist excessive vegetative growth in high input conditions.

A spontaneous brachytic mutant with short internodes and compact growth habit, E100YM, has been identified (Dahiya et al. 1984) and used in ideotype breeding (Dahiya et al. 1988, 1990a, b; Sandhu et al. 1990; Lather 2000). Promising progenies with compact growth habit and which can be grown at high plant density have been obtained (Lather 2000). The mutant E100YM is presently the only source of short internodes and compact growth habit available in chickpea. This report describes an induced mutant that can be used as an alternative source of short internodes and compact growth habit by chickpea breeders.

Materials and methods

Mutations were induced in desi chickpea cultivar JG 315, a wilt-resistant popular variety of central India, through treatment with ethyl methane sulphonate (EMS). The details of seed treatment and growing of M_1 and M_2 generations have been described earlier (Gaur and Gaur 1999, 2003; Gaur et al. 2004). Six major morphological mutants were identified in M_2. Five of these mutants—fasciated stem [Jawahar Gram Mutant 2 (JGM 2)] (Gaur and Gour 1999); broad-few-leaflets (JGM 4) and outwardly curved wings (JGM 5) (Gaur and Gour 2003); and variegated leaf (JGM 3) and apical chlorosis (JGM 6) (Gaur et al. 2004), have been reported earlier. The sixth and the last major mutant (brachytic mutant) obtained from this experiment is described here. This mutant was isolated from M_2 derived from the seed treated with 0.6% EMS for 6 h.

The brachytic mutant was named JGM 1. The mutant was crossed with its parental cultivar JG 315 and two other accessions of desi chickpea, ICC 4929 (double-podded and pink-veined white flower) and ICC 10301 (simple leaf), for study of inheritance of the mutant trait. A spontaneous brachytic growth mutant, E100YM, has been reported earlier by Dahiya et al. (1984). JGM 1 was crossed with E100YM to determine allelic relationship of genes controlling brachytic growth in the two mutants. The F_1 and F_2 were grown in normal field conditions and observations were recorded on each segregating trait on individual F_2 plants. Inheritance and linkage analyses were performed using the computer program LINKAGE-1 (Suiter et al. 1983).

The segregants with compact growth habit and double-pod trait were selected from the cross-JGM 1 (single-podded) × ICC 4929 (double-podded) and single plant progenies were grown in F_3. Further single plant selections were made in F_3. One hundred F_4 progenies, derived from the selected F_3 plants, were evaluated along with 'JG 315.' Each line was grown in a single row of 6 m length, keeping a distance of 30 cm between rows. 'JG 315' was planted after every ten lines. Observations were recorded on ten randomly taken plants in each row.

Results and discussion

The brachytic growth mutant JGM 1 was identified in M_2 of the cultivar JG 315 derived from the seeds treated with 0.6% EMS for 6 h. The mutant had erect and sturdy stem with short internodal and interleaflet distances (Fig. 1). It had few tertiary and later order branches and its seeds were elongated. The mutant bred true in the succeeding generations.

One spontaneous brachytic growth mutant has been earlier reported in chickpea (Dahiya et al. 1984). It was identified from the germplasm line E100Y and was designated E100YM. Although both the mutants had shorter internodes (average internodal distance 1.15 cm in JGM 1 and 0.91 cm in E100YM), closer leaflets, and compact growth habit, they differed from each other for several traits (Table 1; Figs. 1, 2). JGM 1 had less height, lower number of nodes per primary branch, earlier in maturity, higher number of pods per plant, smaller seeds, and higher yield per plant than E100YM. The foliage of E100YM was dark green, whereas JGM 1 had normal green color, most common in chickpea germplasm. The leaves of JGM 1 were straight and more upright, while leaves of E100YM were slightly curved at the terminal end (Fig. 2). The distance between stipule and the first leaflet was higher in JGM 1 as compared to that in E100YM (Fig. 2).

Fig. 1 Plants of spontaneous brachytic growth mutant E100YM (*left*) and induced brachytic growth mutant JGM 1 (*right*)

Inheritance of compact growth habit of JGM 1 was studied in F_2 of three crosses (JGM 1 × JG 315, JGM 1 × ICC 4929, and JGM 1 × ICC 10301). F_1s from all three crosses had normal plant type, indicating the dominance of normal plant type over brachytic plant type. The F_2 population of each cross-gave a good fit to a ratio of 3:1 for normal and brachytic plant types (Table 2). These results suggest that a single recessive gene controls brachytic growth in JGM 1. The brachytic growth in E100YM has also been reported to be controlled by a single recessive gene, designated *br* (Dahiya et al. 1984).

The allelic relationship of genes controlling brachytic growth in JGM 1 and E100YM was studied by intercrossing these mutants. The F_1 from JGM 1 × E100YM had normal plant type, indicating that the genes for brachytic growth in the two mutants are not allelic. The F_2 of the cross-JGM 1 × E100YM produced four types of plants—normal type, JGM 1 type, E100YM type, and Dwarf type in a ratio of 9:3:3:1 (Table 2). These results further confirmed that the genes for brachytic growth in the two mutants were not allelic and thus two genes segregated for brachytic growth in JGM 1 × E100YM cross. We propose to designate the gene for brachytic growth in JGM 1 as *br2* and the *br* gene of E100YM as *br1*.

In F_2 population, the two types of brachytic growth plants (JGM 1 type and E100YM type) could be distinguished based on the leaf characteristics, foliage color, and growth habit of two mutants. The F_2 plants carrying both the brachytic genes in homozygous recessive condition (*br1br1 br2br2* genotype) were very dwarf (Table 3) and had much shorter internodes and closer leaflets than the two mutants. These plants bred true in F_3.

The four plant types obtained in F_2 of JGM 1 × E100YM were compared for plant biomass, plant height, number of pods per plant, number of seeds per plant, 100-seed weight, yield per plant, and harvest index. Significant differences were found among the four plant types for all these traits (Table 3). The normal type plants were taller, produced more number of pods and seeds per plant and gave higher yield than the remaining plant types. JGM 1 type plants were superior to E100YM type plants in productivity (number of pods and seeds per plant and yield per plant) though E100YM type plants had

Table 1 Morphological features of induced brachytic growth mutant JGM 1 and spontaneous brachytic growth mutant E100YM of chickpea

Character	Mean ± SE	
	JGM 1	E100YM
Days to 50% flowering	73 ± 0.84	88 ± 2.68
Days to maturity	126 ± 0.84	135 ± 0.86
Plant height (cm)	28.2 ± 0.96	42.0 ± 0.87
Number of nodes per primary branch	19.6 ± 6.55	30.5 ± 6.81
Average internodal length (cm)	1.15 ± 0.037	0.91 ± 0.001
Number of primary branches per plant	3.4 ± 0.24	3.3 ± 0.24
Number of secondary branches per plant	5.4 ± 0.18	7.1 ± 0.96
Plant dry weight (g)	12.13 ± 1.12	15.20 ± 1.10
Number of filled pods per plant	39 ± 5.96	14 ± 2.56
Number of empty pods per plant	5 ± 0.75	3 ± 0.44
Number of seeds per plant	49 ± 8.73	14 ± 2.72
Seed yield per plant (g)	4.9 ± 0.92	2.8 ± 0.48
100 seed weight (g)	10.8 ± 0.41	20.3 ± 0.58
Harvest index	0.37 ± 0.044	0.18 ± 0.022

Fig. 2 Branches of E100YM (*left*) and JGM 1 (*right*). The leaves of E100YM were curved at the terminal end, while the leaves of JGM 1 were straight and upright

slightly larger seeds. The dwarf type plants had lowest mean values for all the traits.

In addition to *Br2*, two other loci, *Sfl* (one of the two loci involved in controlling number of flower per axis; Srinivasan et al. 2006) and *Ifc* (the recessive allele at this locus inhibits flower color without affecting vein color; Gaur and Gour 2001) segregated in F_2 of JGM 1 × ICC 4929 and one locus *Slv* (the locus that controls simple leaf type; Ekbote 1937) in F_2 of JGM 1 × ICC 10301. The *Br2* locus segregated independently of these loci (data not shown). Thus, it was possible to combine these traits.

The mutant E100YM has been used in developing a plant type with erect and upright growth habit, so that yield per unit area can be increased by enhancing plant density (Dahiya et al. 1988, 1990a, b; Lather 2000; Sandhu et al. 1990). Progenies were obtained which were erect and compact in growth with strong and reasonably tall stem and few but erect secondary and later order branches. One of these lines, H96-99, recorded a yield of about 4.0 t ha^{-1} under high plant density of 50 plants m^2. The plant density generally recommended for chickpea is 33 plants m^2 (Lather 2000).

We found that the compact growth habit plants generally have lower number of pods per plant than the normal plants due to reduction in tertiary and later order of branches. From F_2 of JGM 1 (single-podded) × ICC 4929 (double-podded) cross, we selected plants with compact growth habit with double-pod traits, so that number of pods per plant can be enhanced. F_3 progenies were grown from these plants and 100 single plants were selected based on plant type and number of pods per plant. The F_4 progenies were subjected to preliminary yield evaluation along with the variety 'JG 315.' A comparison

Table 2 Goodness-of-fit χ^2-test for brachytic growth in F_2 of different crosses in chickpea

Cross	Observed frequencies in F_2 phenotypic classes				Expected genetic ratio	χ^2	P
	Normal type	JGM 1 type	E100YM type	Dwarf type			
JGM 1 × JG 315	280	106	–	–	3:1	1.25	0.26
JGM 1 × ICC 4929	180	50	–	–	3:1	1.30	0.25
JGM 1 × ICC 10301	168	44	–	–	3:1	2.04	0.15
JGM 1 × E100YM	200	76	64	20	9:3:3:1	1.55	0.68

Table 3 Morphological features of different plant types observed in F_2 of JGM 1 × E100YM

Character	Mean ± SE			
	Normal type	JGM 1 type	E100YM type	Dwarf type
Plant dry weight (g)	38.9 ± 1.42	23.5 ± 1.59	9.6 ± 1.38	2.8 ± 0.56
Plant height (cm)	43.4 ± 0.54	40.6 ± 0.74	32.5 ± 0.64	21.8 ± 1.99
Number of filled pods per plant	94.6 ± 4.78	69.1 ± 4.96	17.7 ± 2.35	4.5 ± 1.50
Number of empty pods per plant	7.1 ± 0.51	5.6 ± 0.73	2.5 ± 0.41	1.0 ± 0.00
Number of seed per plant	108.1 ± 5.28	74.6 ± 5.17	18.4 ± 2.41	4.0 ± 1.00
Seed yield per plant (g)	17.9 ± 0.89	11.2 ± 0.81	3.35 ± 0.47	0.55 ± 0.25
100-seed weight	16.5 ± 0.32	14.9 ± 0.47	18.6 ± 0.97	13.0 ± 3.00
Harvest index	0.44 ± 0.02	0.44 ± 0.02	0.34 ± 0.03	0.23 ± 0.07

of the performance of the best five lines with the check variety 'JG 315' revealed that these lines were 2–6 days late in maturity, had reduced plant height, more number of pods as well as seeds per plant, at par or higher grain yield per plant and smaller seeds than 'JG 315' (Table 4). A comparison of the morphological features of the check cultivar JG 315 and the new plant types is provided in Figs. 3 and 4. A major improvement in these new plant type lines is needed for seed size and maturity duration. The line ICC 4929 used as source of double-podded trait was not a good choice as it has small seeds and late maturity. The selected lines are now being crossed with early maturing, large seeded and high yielding cultivars for improvement of seed size and reduction in maturity duration.

We expect that the new plant type lines with short internodes will restrict excessive vegetative growth in irrigated conditions. Another trait that could be useful for restricting excessive vegetative growth in chickpea is determinate growth habit. There are reports in other legumes on successful exploitation of this trait in cultivar development, e.g., soybean (Cooper et al. 2004), common bean (Saindon et al. 1996), cowpea (Mligo 1989), mungbean (Sandhu et al. 2003), white lupin (Gataulina et al. 2005), and pea (Scott and Goulden 1993). In soybean, determinate line resisted lodging under high N fertilization, while the indeterminate check cultivar had high lodging, leading to disease infestation, and substantial yield decreases (Wallace et al. 1990). In white lupin, determinate types are better adapted to cool and wet conditions where they are harvestable earlier, and have greater and more stable yields than determinate types (Juller et al. 1993). A mutant with determinate growth habit was identified in chickpea (van Rheenen et al. 1994) but its usefulness could not be assessed as it was sterile. A semi-determinate high yielding and high protein mutant, released as 'Hypersola' in Bangladesh, was found responsive to supplemental N as compared to its indeterminate parental cultivar Faridpur-1 (Shamsuzzamam et al. 2002). More efforts are needed on identifying/inducing determinate genotypes in chickpea and their exploitation in varietal development.

The spontaneous brachytic mutant, E100YM, has been the only source of short internodes and compact growth habit available in chickpea. The induced

Table 4 Performance of the check cultivar JG 315 and best five F_4 new plant type lines (NPT) developed from JGM 1 × ICC 4929 cross

Character	Mean ± SE					
	JG 315	NPT 15	NPT 18	NPT 58	NPT 61	NPT 65
Days to flowering	110 ± 1.3	114 ± 1.6	115 ± 1.8	115 ± 1.4	112 ± 1.0	116 ± 1.5
Plant height (cm)	52.6 ± 2.0	46.0 ± 1.6	42.0 ± 1.5	45.6 ± 1.8	38.2 ± 1.0	41.0 ± 1.3
Number of primary branches per plant	5.5 ± 1.2	6.8 ± 2.1	5.7 ± 1.3	7.2 ± 2.2	6.3 ± 1.4	5.2 ± 1.2
Number of secondary branches per plant	18.1 ± 3.8	16.5 ± 2.4	14.2 ± 2.0	17.4 ± 2.6	14.2 ± 2.3	13.4 ± 2.1
Number of pods per plant	53.0 ± 8.0	93.0 ± 15.2	79.3 ± 9.4	91.8 ± 13.2	76.6 ± 11.6	63.0 ± 9.7
Double-podded nodes (%)	0.0 ± 0.0	19.4 ± 3.0	26.1 ± 4.1	22.4 ± 3.8	27.4 ± 4.3	30.9 ± 4.8
Number of seeds per pod	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.2
Number of seeds per plant	64.0 ± 8.3	102.3 ± 15.6	82.5 ± 10.1	92.7 ± 13.8	84.3 ± 12.2	81.9 ± 10.6
Seed yield per plant (g)	9.2 ± 2.4	11.3 ± 2.3	10.1 ± 2.0	10.8 ± 2.5	9.4 ± 2.3	11.1 ± 2.6
100-seed weight (g)	14.3 ± 0.7	11.0 ± 0.5	12.2 ± 0.6	11.6 ± 0.7	11.1 ± 0.6	13.5 ± 0.9

Fig. 3 Plants of chickpea cultivar JG 315 (*left*) and a new plant type line (*right*) that was developed from a cross-JGM 1 × ICC 4929 and combined compact growth habit of JGM 1 and double-pod trait of ICC 4929

brachytic mutant JGM 1 reported here will provide an alternative source of these desired traits to chickpea breeders. The preliminary results from its utilization as base material in developing an erect plant type with shorter internodes are encouraging. However, further efforts are needed to improve seed size and evaluate the selected lines in different plant density and irrigated conditions.

Fig. 4 Branches of JG 315 (*left*) and a new plant type line (*right*). New plant type lines had a greater number of pods per primary branch

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