Allelic relationships of genes controlling number of flowers per axis in chickpea

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Abstract Genetic variation for number of flowers per axis in chickpea (*Cicer arietinum* L.) includes single-flower, double-flower, triple-flower and multi-flower traits. A double-flowered (DF) line ICC 4929, a triple-flowered (TF) line IPC 99-18 and a multi-flowered (MF) line JGM 7 were intercrossed in all possible combinations and flowering behavior of parents, F_1s and F_2s was studied to establish allelic relationships, penetrance and expressivity of genes controlling number of flowers per axis in chickpea. The F_1 from ICC 4929 (DF) × IPC 99-18 (TF) cross were double-flowered, whereas F_1 from ICC 4929 (DF) × JGM 7 (MF) and IPC 99-18 (TF) × JGM 7 (MF) crosses were single-flowered. The F_2 from ICC 4929 (DF) × IPC 99-18 (TF) cross gave a good fit to a 3:1 ratio for double-flowered and triple-flowered plants. The F_2 from ICC 4929 (DF) × JGM 7 (MF) cross segregated in a ratio of 9:3:3:1 for single-flowered, double-flowered, multi-flowered and double-multi-flowered plants. The F_2 from IPC 99-18 (TF) × JGM 7 (MF) cross segregated in a ratio of 9:3:4 for single-flowered, triple-flowered and multi-flowered plants. The results clearly established that two loci control number of flowers per axis in chickpea. The double-flower and triple-flower traits are controlled by a single-locus (*Sfl*) and the allele for double-flowered trait (sfl^d) is dominant over the allele for triple-flower trait (sfl^t). The three alleles at the *Sfl* locus has the dominance relationship $Sfl > sfl^d > sfl^t$. The multi-flower trait is controlled by a different gene (*cym*). Single-flowered plants have dominant alleles at both the loci (*Sfl_ Cym_*). The double-flower, the triple-flower and the multi-flower traits showed complete penetrance, but variable expressivity. The expressivity was 96.3% for double-flower and 76.4% for double-pod in ICC 4929, 81.2% for triple-flower and 0.0% for triple-pod in IPC 99-18, and 51.3% for multi-flower and 24.7% for multi-pod in JGM 7. Average number of flowers per axis and average number of pods per axis were higher in JGM 7 than double-flowered line ICC 4929 and triple-flowered line IPC 99-18. The results of this study will help in development of breeding strategies for exploitation of these flowering and podding traits in chickpea improvement.

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Introduction

Chickpea (*Cicer arietinum* L.) is the third most important food legume of the world grown in over 40 countries on an area of about 11.2 million hectares, with production of 9.2 million tons and an average yield of 818 kg ha^{-1} (FAOSTAT data, 2006). Chickpea has racemose inflorescence, and most germplasm lines/cultivars produce a single flower at each axis of the raceme. Some germplasm lines/cultivars produce two flowers at several axes, and these are called double-flowered or twin-flowered. Mutants have been identified that produce more than two flowers at several axes. A triple-flowered line, KTP 1, that produces three flowers at several axes (Sindhu and Singh 1987; Singh and Chaturvedi 1998), and a multi-flowered line, JGM 7, that produces more than three flowers at several axes (Gaur and Gour 2002) have been reported. Number of pods per plant and seed size are the most important yield contributing traits in chickpea, and it appears logical to assume that number of pods per axis may have an effect on number of pods per plant and ultimately on grain yield. Thus, there is an interest in exploiting double- and multi-pod traits for developing high yielding cultivars in chickpea.

There are several reports on inheritance of the double-flower (double-pod) trait (Khan and Akhtar 1934; Patil 1966; More and D'Cruz 1976; Yadav et al. 1978; Rao et al. 1980; Pawar and Patil 1983; Singh and van Rheenen 1994; Kumar et al. 2000; Gaur and Gour 2002) and one report each on inheritance of triple-flower (Singh and Chaturvedi 1998) and multi-flower (Gaur and Gour 2002) traits in chickpea. The allelic relationship of genes controlling double-flower and multi-flower traits is also known (Gaur and Gour 2002). However, the allelic relationships of genes controlling the triple-flower, double-flower and multi-flower traits are not known. There are few studies on penetrance and expressivity of genes controlling the double-flower (double-pod) trait, but there are no reports on penetrance and expressivity of genes controlling triple-flower (triple-pod) and multi-flower (multi-pod) traits. Thus, the present study was conducted to study allelic relationships, penetrance and expressivity of genes controlling double-flower (double-pod), triple-flower (triple-pod) and multi-flower (multi-pod) traits in chickpea.

Materials and methods

The experimental materials comprised of three parental lines—ICC 4929 (double-flowered), IPC 99-18 (triple-flowered, TF) and JGM 7 (multi-flowered, MF), six F_1s derived from both direct and reciprocal crosses and three F_2 populations of one-way crosses (ICC 4929 × IPC 99-18, IPC 99-18 × JGM 7 and ICC 4929 × JGM 7) between the three parental lines. ICC 4929 is a breeding line (C 156) collected by ICRISAT from Punjab Province of India. No record is available on its pedigree. This line was selected for use in this study, as it shows high expression of the double-flower trait and was previously used in genetic studies (Gaur and Gour 2002). The genotypes that produce more than two flowers per axis are rare in chickpea. IPC 99-18 is a breeding line developed from a cross between KTP 1 (TF) and KPG 143-4 (SF) at Indian Institute of Pulses Research (IIPR), Kanpur, India. KTP 1 is a spontaneous mutant isolated from single-flowered line K 850 (Sindhu and Singh 1987). IPC-99-18 was used as source of triple-flower trait in this study, as seed of KTP 1 was not available. JGM 7 is a mutant line developed at Jawaharlal Nehru Agricultural University, Jabalpur, India from a spontaneous mutant isolated from an interspecific cross between the accession ICC 5783 (SF) of *Cicer arietinum* and the accession ICCW 9 (SF) of *C. reticulatum* (Gaur and Gour 2002). This mutant is registered (INGR No. 03064) with National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India.

There was wide variation between the parental genotypes for phenology (days to 50% flowering and days to maturity), flower color, stem pigmentation, number of flowers per axis, 100-seed weight and resistance to fusarium wilt (Table 1). One or more of these traits were used to confirm the hybridism of F_1 plants.

The trial was sown during post-rainy season 2004/05 at two locations of India—one in southern part (Patancheru in Andhra Pradesh

Table 1 Characteristics of parental genotypes

Characteristic	ICC 4929	IPC 99-18	JGM 7
Growth habit	Semi erect	Semi erect	Semi spread
Flower color	Pink-veined white	Pink	Pink
Pigmentation on stem	No	Light	Dark
Foliage color	Normal green	Light green	Bronze or purple
Flowering type	Double-flowered	Triple-flowered	Multi-flowered
Days to 50% flowering	74	59	46
Days to maturity	128	115	114
100-seed weight (g)	11.2	17.2	12.4
Seed type	Desi	Desi	Desi
Seed coat color	Yellow brown	Yellow brown	Yellow brown
Reaction to Fusarium wilt	Susceptible	Resistant	Susceptible

province) and the other in northern part (Kanpur in Utter Pradesh province). The trial conducted at Kanpur had to be discarded as over 50% plants died due to fusarium wilt disease. Thus, results from Patancheru location are presented here. Patancheru is located at an altitude of 545 m above the mean sea level, at a latitude of $17^{\circ}27'$N and at a longitude of $78^{\circ}28''$E. The soil of the experimental area was vertisol. The sowing was done during second week of October 2004 keeping row-to-row spacing of 60 cm and plant-to-plant spacing of 20 cm. One irrigation was provided during early flowering stage to overcome moisture stress. The standard package of practices was followed to raise a healthy crop. Observations were recorded on 40 plants in each parental line for calculating penetrance and expressivity of traits, and a minimum of 330 plants in each F_2 population for the study of inheritance and allelic relationships of genes controlling these traits.

The F_2 data was analyzed for determining inheritance of traits using the computer program "LINKAGE-1" (Suiter et al. 1983), which tests goodness-of-fit to expected ratios by chi-square analysis. Penetrance and expressivity of genes controlling flower number per axis were determined for the parental genotypes. The observation on number of flowers at each node was difficult on large number of plants because of indeterminate growth habit of chickpea. Penetrance was calculated as percentage of plants showing that particular trait (double-flower, triple-flower or multi-flower), while expressivity was calculated as percentage of flowering nodes showing that trait.

Results and discussions

Chickpea flowers are typically papilionaceous and emerge on the axillary raceme by a peduncle which is divided into two parts from an arista. The part attached to the leaf axis is called racemal peduncle, while the part attached to the flower is called floral peduncle. Most germplasm lines/cultivars produce a single flower per axis (Fig. 1a, e), and a small proportion ($< 0.1\%$) of germplasm lines/cultivars produce two flowers at several axes (e.g. ICC 4929, Fig. 1b, f). In double-flowered genotypes two floral peduncles are attached to one racemal peduncle. Thus, there is always one racemal peduncle per axis, while the number of floral peduncles may vary depending on number of flowers per axis. The genotypes that produce more than two flowers per axis are rare in chickpea. One mutant KTP 1 has been described to produce three flowers at several nodes (Sindhu and Singh 1987; Singh and Chaturvedi 1998) and a triple-flowered breeding line IPC 99-18 (Fig. 1c, g), derived from a cross involving KTP 1 as one of the parents, was used in this study. The multi-flower line JGM 7 (Fig. 1d, h) used in this study is the only line known to produce more than three flowers at several axes (Gaur and Gour 2002).

Inheritance and allelic relationships

All F_1s (direct and reciprocal) from the cross between ICC 4929 (DF) and IPC 99-18 (TF) were double-flowered, and the F_2 segregated for double-flowered and triple-flowered plants in a ratio of 3:1 (Table 1), suggesting that a single gene controls this trait and the allele for double-flower

Fig. 1 A branch of (**a**) a single-flowered line (most common in occurrence) (**b**) the double-flowered line ICC 4929 (**c**) the triple-flowered line IPC 99-18, and (**d**) the multi-flowered line JGM 7; (**e**) single-flower (**f**) double-flower (**g**) triple-flower, and (**h**) multi-flower traits exhibited by the above genotypes, respectively
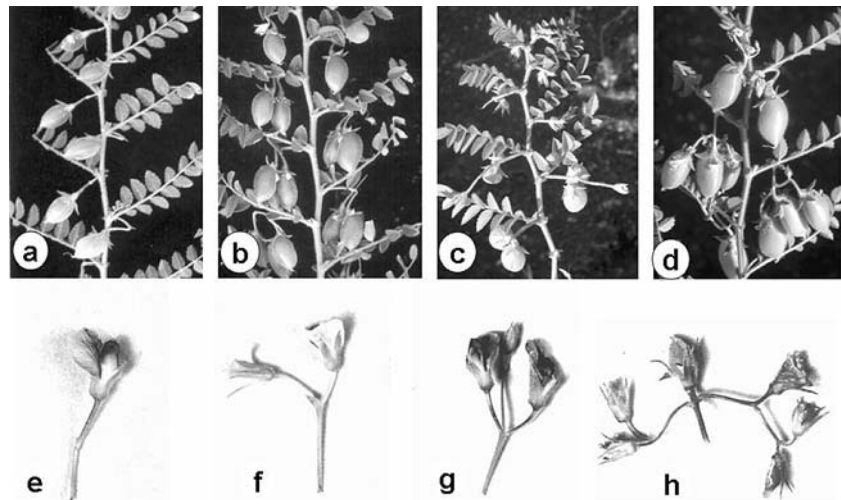
trait is dominant over that for triple-flower trait. There are several reports (Khan and Akhtar 1934; Patil 1966; More and D'Cruz 1976; D'Cruz and Tendulkar 1970; Yadav et al. 1978; Rao et al. 1980; Pawar and Patil 1983; Singh and van Rheenen 1994; Kumar et al. 2000; Gaur and Gour 2002) on inheritance of double-flower (double-pod) trait in chickpea, which suggest that single-flower (single-pod) trait is dominant over double-flower (double-pod) trait and controlled by a single locus. The recessive gene for double-flower trait was designated *s* by Khan and Akhtar (1934) and *sfl* by D'Cruz and Tendulkar (1970). Though both gene symbols have been used in literature, we prefer to use *sfl* as most gene symbols in chickpea have a triple alphacode. The inheritance of the triple-flower trait was studied by Singh and Chaturvedi (1998), from crosses of a spontaneous triple-flowered mutant KTP 1 with five single-flowered lines. They found that the triple-flower trait is controlled by a single locus, and suggested a gene symbol *rtf* for the gene controlling triple-flower trait. The allelic relationship between genes controlling double-flower and triple-flower traits has been studied for the first time in this study. The results clearly showed that the genes for double-flower and triple-flower traits are allelic. Thus, there is a need to change the gene symbols for these traits. Following the nomenclature guidelines for *Pisum* gene symbols (Blixt 1977), we suggest sfl^d for the allele for the double-flower trait and sfl^t for the allele for the

triple-flower trait. The three alleles identified at *Sfl* locus has the dominance relationship $Sfl > sfl^d > sfl^t$.

Gaur and Gour (2002) studied inheritance and allelic relations of genes controlling double-flower and multi-flower traits from the cross ICC 4929 × JGM 7. They suggested a gene symbol *cym* for the gene controlling the multi-flower trait, as the flowers are arranged in a cymose inflorescence at each axis of the raceme in the multi-flowered line. Their results indicated that the gene for the double flower trait is not allelic to the gene for multi-flower trait. Thus, JGM 7 has the genotype *Sfl Sfl cym cym*. The results of this study are in agreement with their results. All F_1s from direct and reciprocal crosses between double-flowered line ICC 4929 (sfl^d sfl^d *Cym Cym*) and multi-flowered line JGM 7 (*Sfl Sfl cym cym*) were single-flowered (*Sfl* sfl^d *Cym cym*). The F_2 segregated in a ratio of 9 single-flowered (*Sfl _ Cym_*), 3 double-flowered (sfl^d sfl^d *Cym_*), 3 multi-flowered (*Sfl _ cym cym*), and 1 double-multi-flowered (sfl^d sfl^d *cym cym*) plants (Table 2). It was possible to differentiate between multi-flowered and double-multi-flowered plants, based on number of floral peduncles attached to a racemal peduncle. Two floral peduncles are attached to a racemal peduncle in case of double-flower and double-multi-flower types, while three floral peduncles are attached to a racemal peduncle in multi-flower type.

Table 2 Goodness-of-fit χ^2 test for F_2 segregation of different flowering types in chickpea

Cross	Phenotype of F_1	Observed frequencies in F_2 phenotypic classes[a]						Expected genetic ratio	χ^2	P
		SF	DF	TF	MF	DMF	Total			
ICC 4929 × IPC 99-18	Double-flowered	–	254	78	–	–	332	3:1	0.40	0.53
ICC 4929 × JGM 7	Single-flowered	195	66	–	72	27	360	9:3:3:1	1.51	0.71
IPC 99-18 × JGM 7	Single-flowered	199	–	74	87	–	360	9:3:4	0.13	0.71

[a] SF, Single flowered; DF, double flowered; TF, triple flowered; MF, multi-flowered; DMF, double-multi-flowered

The allelic relationship between the genes controlling the triple-flower trait (sfl^t) and the multi-flower trait (cym) has been studied for the first time in the present investigation. The F_1s from direct and reciprocal crosses between triple-flowered line IPC 99-18 ($sfl^t\ sfl^t\ Cym\ Cym$), and multi-flowered line JGM 7 ($Sfl\ Sfl\ cym\ cym$) were single-flowered ($Sfl\ sfl^t\ Cym\ cym$). The F_2 gave a good fit to a 9:3:4 ratio for single-flowered ($Sfl\ \_\ Cym\_$), triple-flowered ($sfl^t\ sfl^tCym\_$), and multi-flowered plants ($Sfl\ \_\ cym\ cym,\ sfl^t\ sfl^t\ cym\ cym$) (Table 2). It was not possible to distinguish the double recessive class $sfl^t\ sfl^t\ cym\ cym$ (triple-multi-flowered) from multi-flowered ($Sfl\ \_\ cym\ cym$), as in both these classes three peduncles are attached to a racemal peduncle, so these two classes were combined. This led to an expected ratio of 9:3:4 in place of 9:3:3:1. These results further confirm that two genes are involved in controlling triple-flower and multi-flower traits. These results are expected as the gene for triple-flower trait is allelic to the gene for the double-flower trait which is not allelic to gene for multi-flower trait.

Penetrance and expressivity

The penetrance of double-flower, triple-flower and multi-flower traits was studied in ICC 4929 (DF), IPC 99-18 (TF) and JGM 7 (MF), respectively. Complete penetrance was observed for all three flowering types in respective parental lines. That means all plants of ICC 4929 expressed double-flower trait, all plants of IPC 99-18 expressed the triple-flower trait, and all plants of JGM 7 expressed the multi-flower trait. However, the expressivity (percentage of flowering nodes showing the trait in a plant) varied considerably

from one flowering type to another (Table 3). The double-flowered line ICC 4929 showed a mean expressivity of 96.3% for the double-flower trait and the remaining flowering nodes had solitary flowers. Only one or two flowering nodes in a few plants produced three flowers and none produced more than three flowers.

The expressivity of the triple-flower trait was found to be 81.2% in IPC 99-18, and each of the remaining flowering nodes had one or two flowers (Table 3). More than three flowers at a flowering node were not observed in this genotype. The multi-flowered line JGM 7 produced one to seven flowers per flowering node and the expressivity of multi-flower trait (three or more flowers) was 51.3%. It produced one, two, three, four, five, six and seven flowers at 31.3, 17.4, 23.5, 9.9, 11.4, 4.0, and 2.5% of flowering nodes, respectively. The average number of flowers per flowering node was 1.96 in double-flowered line ICC 4929, 2.80 in triple-flowered line IPC 99-18 and 2.83 in multi-flowered line JGM 7.

Usually all flowers in chickpea do not set pods. The environmental conditions, particularly extremes of temperatures, greatly influence fertilization, pod set and seed development. On average, 79.26% of the flowering nodes produced pods in the double-podded line ICC 4929, and 76.4% of the podding nodes had two pods (Table 3). Surprisingly, the triple-flowered line IPC 99-18 did not produce three pods. One flower, which was distinctly different in appearance than the other two flowers at each flowering node (Fig. 1c, g), always failed to set pods. Thus, in IPC 99-18, 55.3% of the podding nodes had double pods and the remaining podding nodes had single pods. In multi-flowered line JGM 7, 42.2% of podding nodes had single pod, 33.0%

Table 3 Expression of number of flowers and number of pods per axis in parental lines

	ICC 4929		IPC 99-18		JGM 7	
	Mean	Range	Mean	Range	Mean	Range
Percentage of flowering nodes with						
1 flower	3.7	0.0–40.0	–	–	31.3	0.0–55.6
2 flowers	96.3	60.0–100.0	18.8	0.0–80.0	17.4	0.0–42.9
3 flowers	–	–	81.2	20.0–100.0	23.5	10.0–44.4
4 flowers	–	–	–	–	9.9	0.0–25.0
5 flowers	–	–	–	–	11.4	0.0–40.0
6 flowers	–	–	–	–	4.0	0.0–20.0
7 flowers	–	–	–	–	2.5	0.0–20.0
Average number of flowers per node	1.96	1.70–2.00	2.8	1.90–3.00	2.83	1.2–4.1
Percentage of podding nodes with						
1 pod	23.6	10.0–80.0	44.7	10.0–100.0	42.2	16.7–71.4
2 pods	76.4	20.0–90.0	55.3	0.0–77.8	33.0	0.0–83.3
3 pods	–	–	0.0	–	17.6	0.0–66.7
4 pods	–	–	–	–	7.2	0.0–40.0
>4 pods	–	–	–	–	–	–
Average number of pods per podding node	1.77	1.70–1.90	1.44	0.70–1.90	2.16	0.88–4.17

had two pods, 17.6% had three pods and 7.2% had four pods. More than four pods were not observed. The average number of pods per podding node was 1.77 in double-flowered line ICC 4929, 1.44 in triple-flowered line IPC 99-18 and 2.16 in multi-flowered line JGM 7.

Kumar et al. (2000) determined penetrance of genes controlling the double-flower trait based on expression of double-pod trait. They found 100% penetrance in parent, 53% penetrance in F_2, and 84.5% in recombinant inbred lines (RILs) of the cross ICCV 2 (single-flowered) × JG 62 (double-flowered). The expressivity of double-pod trait varied from 1.1 to 14.8% in F_2 and 0.1 to 3.3% in RILs. They further found that the late sowing could enhance expression of double-pod trait. Thus, the expressivity of a trait can be manipulated by changing the genetic background and the environmental conditions.

There are variable reports on yield advantages of the double-pod trait in chickpea. Sheldrake et al. (1978) observed an yield advantage of 6–11% under conditions in which double-pod trait is well expressed. Singh and van Rheenen (1989, 1994) observed higher mean yields of double-podded plants than that of single-podded plants. Kumar et al. (2000) found an yield advantage of 18% in F_2 and 7% in RILs due to the double-pod trait. However, no significant effect of the double-pod trait on yield contributing characters was

observed by Knights (1987). Rubio et al. (1998) also did not find any yield increase due to double-pod trait but this trait had a positive effect on the stability of yield.

Exploitation of double- and multi-pod traits appears promising for improving and stabilizing chickpea yields. The information on inheritance and allelic relationships of the genes controlling these traits obtained in the present study would be useful in development of breeding strategies for exploitation of these traits. This study showed that two loci, *Sfl* and *Cym*, control number of flowers per axis in chickpea. The single-flowered genotypes have dominant alleles at both the loci (*Sfl_ Cym_*). The homozygous recessive condition at *Cym* locus (*cym cym*) is needed for expression of multi-flower trait. Multiple alleles were identified at the *Sfl* locus with the dominance relationship *Sfl* (single-flower) > *sfl^d* (double-flower) > *sfl^t* (triple-flower). New combination of traits could be obtained by making different combination of alleles at the two loci. For example, plants with *sfl^d sfl^d cym cym* genotype produce double-multi-flower, and with *sfl^t sfl^t cym cym* genotype produce triple-multi-flower. There is a need for development of advanced breeding lines with different gene combinations and evaluate these in different environments. It should be possible to enhance expression of these traits by using different genetic backgrounds and also by

providing the favorable environmental conditions for the expression of the trait.

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