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Cytogenetic analysis of a novel yellow flower mutant carrying a reciprocal translocation in grass pea (*Lathyrus sativus* L.)

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A rare yellow flower mutation (*Yfm*) was isolated in 200 Gy gamma ray irradiated M_2 progeny of grass pea cv. BioR-231. Except contrasting flower colour, the mutant plants were morphologically indistinguishable from their mother variety. Inheritance studies and allelism test revealed that the mutation was monogenic recessive to normal blue flower and true breeding for its flower colour phenotype. A thorough cytogenetic analysis for several generations pointed out that *Yfm* was semisterile, and was actually heterozygous for a reciprocal translocation (RT). Occurrence of a ring of four chromosomes (1IV + 5II) at diakinesis and metaphase I confirmed the presence of translocation in the mutant plants. RT transmitted at an average of 47.24% in selfed progeny and of 45.56% in intercrossed progeny. Presence of two rings of four chromosomes (2IV+3II) at meiosis I in F_1 progeny obtained from intercrosses between *Yfm* and five previously described RT lines (RT1-5) confirmed independent nature of the present RT line, and thus, it was designated as RT-6. Primary trisomic analysis and linkage study put *Yfm* on extra chromosome of trisomic VI with an estimated map distance of 10.31 cM from the translocation breakpoint.

Key words: cytogenetics, yellow flower mutation, reciprocal translocation, *Lathyrus sativus* L.

INTRODUCTION

Reciprocal translocations, also known as chromosomal interchanges, arise from the exchange of broken segments of non-homologous chromosomes, and are the most frequent types of mutations induced by radiation in different crop plants (Burnham, 1962; Sjödin, 1971; Sadanaga & Newhouse, 1982; Singh, 2003). Thus, an individual with a reciprocal translocation contains a segment of one linkage group attached to another linkage group, and the breakpoint is the point of attachment of the broken segments (Mahama & Palmer, 2003). A translocation heterozygote exhibits partial pollen and ovule sterility (40-60% sterility), and this character defines the translocation breakpoint which shows linkage with mutations of the two different linkage groups (Mahama & Palmer, 2003; Singh, 2003). Associations between different genetic markers and translocation breakpoint were reported in soybean (Sacks & Sadanaga, 1984), lentil (Tadmor *et al*., 1987), pea (Gorel *et al*., 1992) and in other non-leguminous crops (Figueiras *et al*., 1985).

Grass pea (*Lathyrus sativus* L.) is an annual legume crop with 2n = 2x = 14 chromosomes in its somatic cells. The flowers are typically papilionaceous comprising of one large standard, two laterals (aloe or wing) and two innermost petals or keels. Flower colour has been considered as an important descriptor of this crop for germplasm evaluation and classification (Campbell, 1997). The blue-flowered accessions are predominant in Indian subcontinent, while white or mixed colour types have a more western distribution, around the Mediterranean region (Jackson & Yunus, 1984; Campbell, 1997). Low genetic variability and inter-specific incompatibility have hindered its improvement as a pulse crop by conventional breeding methods. In this background, induced mutagenesis has been successfully used to create additional genetic variations in existing germplasms of grass pea. In recent years, progress has been made in

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linkage mapping of different mutations through the development of different cytogenetic tools including trisomics, tetrasomics and polyploid lines (Talukdar, 2008, 2009b, c, 2010b). Along with different aneuploids and other structural chromosomal aberrations, reciprocal translocation served as excellent cytogenetic tool for identification and mapping of different linkage groups in plants (Sybenga, 1996). It has also been involved successfully in transferring desirable traits (Sears, 1956; Gustafsson, 1965) and to generate different trisomics in a number of crop plants (Pellew, 1940; Ramage, 1960; Ashraf & Bassett, 1987; Auger & Birchler, 2002).

During the ongoing research on radiation induced different cytogenetic stocks in the present author's laboratory, two variant plants were identified and isolated by their characteristic yellow flower, a distinct variation from normal blue flower, in 200 Gy gamma ray treated M_2 progeny of grass pea var. BioR-231. Initial cytological analysis pointed out that the two plants were semi-sterile (pollen sterility 46.70%) and heterozygous for a reciprocal translocation in their diploid complement. After necessary confirmation in advanced selfed generations, they were tentatively designated as RT-6. This is in addition to existing five different types of RTs (RT 1-5) maintained by the author in *Lathyrus sativus* L (Talukdar, 2010a; unpublished data). The yellow flower mutation (*Yfm*) might have association with translocation or not, and the chromosomes involved in interchanges in RT-6 line might be the same or different to the existing five RTs. The present investigation was, therefore, carried out to 1) explore the genetic basis of *Yfm*, and its possible chromosome location, 2) analyse the transmission pattern and cytogenetics of RT-6 in selfed and intercrossed progenies, 3) map the *Yfm* with translocation breakpoint, and finally, 4) to test the independence of this newly found translocation, that is, whether RT-6 carried common or different translocated chromosomes with the five other translocation lines in *L. sativus* L. The term 'RT' for reciprocal translocation will be used in this communication.

MATERIALS AND METHODS

Origin of RT-6 mutation

Fresh and dry seeds of grass pea variety BioR-231 were treated with different doses (50, 100, 150, 200, 250, 300, 350 and 400 Gy) of gamma rays to induce mutations. M_1 seeds were sown treatment-wise in a randomized block design keeping uniform distances of 20 cm and 30 cm between plants and rows, respectively, to raise M_2 progenies. Untreated mother variety BioR-231 was used as control throughout the experiment. In M_2 generation, two variant plants in 200 Gy irradiated progeny were identified by contrasting modification of normal blue flower into characteristic yellow-coloured petals in flower. The plants were, otherwise, looking normal. However, cytological studies revealed occurrence of typical multivalent association at meiosis I and partial pollen sterility (46.70%). These two plants were selfed for three successive generations at an experimental farm in Kalyani (22°59′ N, 88°29′ E), West Bengal, India, and their progeny plants (M_3-M_5) with characteristic yellow flower were cytologically confirmed as heterozygous for a translocation. On the basis of their completely different flower colour, they were tentatively designated as RT-6 to distinguish it from rest of the five lines (RT 1-5) characterized earlier in grass pea (Talukdar, 2010a, unpublished data).

Inheritance studies of yellow flower mutation (Yfm) and test of allelism

To trace the mode of inheritance of *Yfm*, reciprocal crosses were made between this mutant (M_5) and the control variety BioR-231. Three other varieties, namely, BioL-203, Hooghly Local (HL) and B1 were also included in the crossing programmes. All the four varieties were blue-flowered genotypes. F_1 progeny was raised in each cross during winter season of 2006-2007. F_2 population was grown in subsequent season. Test cross between F_1 and recessive phenotype was also performed. Segregation data were tested by χ^2 to determine goodness-of-fit between observed and expected ratios (Table 1). Necessary cytological studies of each progeny plant in F_1, F_2 and test cross generations were performed at meiosis I stage.

In order to ascertain the allelic relationship of gene(s) controlling yellow flower with other flower colour mutations, three induced mutant lines, namely, pale-violet flower mutation (PVFM), white flower mutation (WFM) and blue-patched white flower mutation (BPWFM) were incorporated in the present study. All the three lines were isolated in gamma ray irradiated progeny of different grass pea varieties, and have been maintained for several generations by selfing as true breeding mutant lines without showing any type of chromosomal abnormalities (Talukdar *et al*., 2002; Talukdar & Biswas, 2006, 2007a; Talukdar, 2009b). Direct as well as reciprocal crosses were ma-

TABLE 1. Inheritance and allelic relationship of yellow flower mutation (*Yfm*) in grass pea (*Lathyrus sativus* L.). HL[a]: Hooghly Local; BPWFM[b]: blue-patched white flower mutant; WFM[c]: white flower mutant; PVFM[d]: pale-violet flower mutant

Cross	F_1	F_2/test cross segregation		total	χ^{2*}	*p*
	phenotype	blue	yellow		(3:1/1:1)	
Yfm × BioR-231	blue	69	22	91	0.03	0.90-0.80
F_1 × *Yfm*	–	33	30	63	0.14	0.80-0.70
Yfm × BioL-203	blue	54	20	74	0.16	0.70-0.50
F_1 × *Yfm*	–	21	25	46	0.35	0.70-0.50
Yfm × B1	blue	59	17	76	0.28	0.70-0.50
F_1 × *Yfm*	–	31	27	58	0.28	0.70-0.50
Yfm × HL[a]	blue	63	19	82	0.15	0.70-0.50
F_1 × *Yfm*	–	36	29	65	0.75	0.50-0.30
Total (F_2)	blue	245	78	323	0.12	0.80-0.70
Total (test cross)	–	121	111	232	0.43	0.70-0.50
Inter-mutant crosses		yellow	blue-patched white/white			
Yfm × BPWFM[b]	yellow	101	32	133	0.06	0.90-0.80
F_1 × BPWFM[b]	–	43	39	82	0.20	0.70-0.50
Yfm × WFM[c]	yellow	55	20	75	0.11	0.80-0.70
F_1 × WFM[c]	–	23	19	42	0.38	0.70-0.50
Yfm × PVFM[d]		yellowish -brown	pale yellow -violet	white	χ^{2*} (9:3:3:1)	*p*
yellowish-brown	135	43	39	16	0.78	0.90-0.80

* consistent with respective ratio at 5% level

de between yellow flower mutation and the three mutant lines during winter season of 2007-2008. The F_1, F_2 and back cross generations were evaluated in normal field condition.

Chromosomal location of yellow flower mutation through primary trisomic analysis

Chromosomal localization of a gene was determined on the basis of the modified F_2 segregation ratios in primary trisomics following the methods adopted earlier in different crops including grass pea (Hermsen, 1970; Khush *et al*., 1984; Talukdar, 2009a). This is based on the concept that frequency of recessive phenotype in diploid F_2 progeny coming from the original mutant line × critical trisomic line (bearing particular gene/mutation) greatly differs with the frequency derived from the original mutant line x other trisomic line (not bearing that gene/mutation). In the last couple of years, detail crossing programmes were undertaken between *Yfm* and seven different primary trisomics (Tr I-VII), isolated and characterized in grass pea (Talukdar & Biswas, 2007b). Crosses were made between each of the trisomics as the female parent and the mutant line as the male parent during winter of 2006-2007. F_1 plant from each of the crosses was harvested individually. On the basis of unique leaflet and stipule characters, different trisomic phenotypes in segregating populations could be readily identified at seedling stages, and their chromosome numbers were confirmed at meiotic metaphase-I (Talukdar & Biswas, 2007b). F_1 plants showing disomic phenotype ($2n = 14$) were also identified by meiotic analysis. Trisomic F_2 plants were recovered from the selfed progeny of F_1 in the following season. BC_1 population was raised by crossing trisomic F_1 with recessive mutant parent. Meiotic chromosome association was studied in F_2 recessive homozygotes and BC_1 plants. In the segregating F_2 and BC_1 progenies, disomic and trisomic plants in different crosses were classified each as dominant phenotype of normal blue flower and recessive mutant phenotype of yellow flower. Segregation of normal and mutant phenotypes in diploid portion of total population was examined by means of χ^2 test for disomic segregation ratio of

TABLE 2. Segregation of blue and yellow flower in F_2 and BC_1 generations of crosses between *Yfm* and seven different primary trisomics (Tr I-VII) of grass pea (*Lathyrus sativus* L.). *, ** and *** significant at 0.05, 0.01 and at 0.001 level of probability, respectively

Trisomic F_1	F_2 and BC_1 phenotype									
	2n		Total	χ^2				2n+1		
	Blue	Yellow		(1:1)	(2:1)	(3:1)	(8:1)	Blue	Yellow	Total
(*Yfm* × Tr I) selfed	71	23	94	–	–	0.01	16.99***	19	07	26
(*Yfm* × Tr I) × *Yfm*	41	38	79	0.11	7.76***	–	–	07	01	08
(*Yfm* × Tr II) selfed	102	31	133	–	–	0.20	20.03***	21	09	30
(*Yfm* × Tr II) × *Yfm*	52	47	99	0.25	8.91*	–	–	09	03	12
(*Yfm* × Tr III) selfed	69	22	91	–	–	0.03	15.73***	57	19	76
(*Yfm* × Tr III) × *Yfm*	29	25	54	0.30	4.08*	–	–	14	07	21
(*Yfm* × Tr IV) selfed	129	40	169	–	–	0.16	27.01***	55	13	68
(*Yfm* × Tr IV) × *Yfm*	67	59	126	0.51	10.32**	–	–	10	05	15
(*Yfm* × Tr V) selfed	90	34	124	–	–	0.36	33.43***	77	17	94
(*Yfm* × Tr V) *Yfm*	44	37	81	0.60	5.55*	–	–	13	05	18
(*Yfm* × Tr VI) selfed	159	21	180	–	–	17.07***	0.06	17	00	17
(*Yfm* × Tr VI) *Yfm*	71	37	108	10.70**	0.04	–	–	10	00	10
(*Yfm* × Tr VII) selfed	87	27	114	–	–	0.10	18.23***	27	09	36
(*Yfm* × Tr VII) *Yfm*	31	26	57	0.44	3.87*	–	–	12	05	17

3:1 in F_2 and 1:1 for test cross. Significant deviation from this ratio was again tested for 8:1 in F_2 and 2:1 in test cross in diploid portion of population. The modified segregation ratio along with frequency of recessive phenotype in the diploid as well as in trisomic progeny was used to locate possible trisomic chromosome bearing gene for mutation (Table 2).

Segregation of translocation heterozygote in selfed and intercrossed progeny

The control variety BioR-231 and the other three grass pea varieties, BioL-203, HL and B1 were fully fertile and normal showing complete absence of chromosomal aberration. These four parents were classified as homozygous for normal chromosomal arrangement. Inheritance of translocation heterozygosity was traced in selfed progeny (three successive generations) of RT-6 as well as in intercrossed populations of RT-6 and the four varieties. Standard nomenclature of N/N for homozygous normal nontranslocation chromosomes, N/T for heterozygous translocation, and T/T for homozygous translocation chromosome was used as followed earlier (Talukdar, 2010a). The occurrence of quadrivalent was interpreted as evidence of translocation heterozygosity in the present study. In the selfed progeny of RT-6, both fertile and semi-sterile plants appeared. Among the fertile plant population, some of the plants exhibited weak growth habit in comparison to other, but cytological studies revealed presence of 7 bivalents at metaphase I in microsporocytes of all fertile plants (N/N and T/T). The plants showing weak growth were separately harvested. To distinguish N/N plants from T/T in fertile plant population, plants with normal growth habit and with weak growth were crossed separately with control variety BioR-231 to obtain F_1 progeny. Chromosome configurations at metaphase I and pollen sterility were analyzed in these F_1 plants. The plants that produced F_1 with multivalent association and semisterile pollen after crossing with control variety were categorized as T/T plants. Plants that produced completely fertile F_1 offsprings when crossed with control variety were classified as homozygous for normal chromosomal arrangement (N/N).

Segregation of N/T and other plant types in the progeny were studied in intercrossed population of RT-6 and four parents, BioR-231, BioL-203, HL and B1. Chi-square test was employed in each case to test the segregating selfed progeny of RT-6 for the expected ratio of 1(N/N):2 (N/T):1 (T/T) and again for

TABLE 3. Joint segregation and linkage study of genes controlling yellow flower (*Yfm*) and a translocation breakpoint in F_2 and test cross progenies of four nontranslocation varieties and RT-6 line of *Lathyrus sativus* L.

Cross	F_2 and test cross segregations				Total	χ^{2**}		cov%	map distance (cM)
	XY	Xy	xY	xy		(9:3:3:1)	(1:1:1:1)		
RT-6 × BioR-231	101	36	49	71	257	204.16	–	–	–
F_1 × RT-6	62	08	05	50	125	–	80.86	10.40	10.55
RT-6 × BioL-203	98	15	20	87	220	423.23	–	–	–
F_1 × RT-6	65	07	06	55	133	–	87.60	10.00	10.14
RT-6 × HL*	159	19	11	138	327	750.01	–	–	–
F_1 ×RT-6	52	07	03	36	98	–	67.64	10.20	10.35
RT-6 × B1	101	11	33	90	235	421.05	–	–	–
F_1 × RT-6	61	06	06	43	116	–	78.55	10.30	10.45
Pooled test cross	240	28	20	184	472	–	313.08	10.17	10.31

*HL-Hooghly Local, ** significant at 0.05 level

TABLE 4. Metaphase I chromosome configuration of F_1 plants derived from RT × normal cultivars and intercrosses between RT-6 and five other RT lines (RT1-5) in grass pea (2n = 14)

	Metaphase I chromosome associations in the progeny					Total plants
Cross	7II	1IV+5II	2IV+3II	1VI+4II	pentavalent	
RT-6 × BioR-231	36	31	–	–	03	70*
RT-6 × BioL-203	34	30	–	–	02	66*
RT-6 × HL	35	29	–	–	03	67*
RT-6 × B1	32	33	–	–	02	67*
Total (RT-6 × variety)	137	123	–	–	10	270
RT-6 × RT-1	67	32	23	–	–	122
RT-6 × RT-2	64	35	31	–	–	130
RT-6 × RT-3	59	43	17	–	–	119
RT-6 × RT-4	67	41	19	–	–	127
RT-6 × RT-5	80	20	12	–	–	112
Total (RT-6 × RT 1-5)	337	171	102	–	–	610

* Segregation of 7II and 1IV + 5II (N/T plants) consistent with the expected 1:1 ratio at 5% level of significance with χ^2 value (df = 1) 0.37 in RT-6 × BioR-231, 0.25 in RT-6 × BioL-203, 0.56 in RT-6 × HL, and 0.02 in RT-6 × B1

the ratio of 1 fertile: 1 semisterile in the progeny of normal plants × RT-6 line (Table 3). Meiotic I chromosome association was studied to detect aneuploids in both selfed and intercrossed progeny.

Mapping yellow flower mutation and translocation breakpoint

Map distance between loci controlling yellow flower mutation and translocation breakpoint was determined by linkage association between them in offspring derived from crosses between RT-6 and non translocation (N/N) lines (four varieties). The RT plants were identified by semi-sterile pollen and 1IV+ 5II chromosome association at meiosis I, while plants with normal pollen fertility, showing uniform presence of 7II at meiosis I, were detected as N/N lines. In linkage tests, the inheritance of the translocation based semisterility was treated as being caused by a locus. Meio-

tic analysis and pollen fertility of all the F_1 plants from RT-6 × N/N were carried out, and F_1 plants were backcrossed to recessive parent. Map distance between two loci was calculated by putting cross over value in percentage (cov%) obtained from test cross segregations in Kosambi's mapping function (Kosambi, 1944). Test cross data were pooled when homogeneous (Table 3).

Test of independence of RT-6

In order to test the independence of the present RT-6 line with the other five RTs, the RT-6 line was crossed with each of them (Table 4). If the two structural heterozygotes involved in the crosses carried the same translocation, their common offspring would show a 1:1 ratio of homozygotes (normal or translocation) and single heterozygous plants. When 2 translocations were different, double heterozygotes would be expected in their offspring. Multivalent configurations exhibited by double heterozygotes at meiosis I in the intercrossed progeny have been used as criteria to ascertain the involvement of common or different chromosomes in the present RT-6 line. If the hybrid plant carried a ring of six chromosomes, then the 2 translocations involved in the crosses should have one chromosome in common, whereas formation of two rings or chains of 4 chromosomes at metaphase I would indicate absence of common chromosome between the translocation lines involved in the crosses (Burnham *et al*., 1954). Presence of 1IV + 5II, however, is the indication of same translocation carried by two translocation heterozygote parents.

Cytological analysis

Diakinesis and metaphase I chromosome configurations have been mainly used to distinguish plants carrying RT and normal nontranslocated chromosomes in selfed and intercrossed progenies of RT-6 line. At flowering, suitable-sized flower buds were collected from each plant between 9:30 AM and 11.00 AM and fixed in freshly prepared 1:3 mixture of acetic acid and ethanol for 24 hrs at room temperature. After 24 hrs, the fixative was replaced with 70% ethanol, and samples were stored at 4°C for further observation. Anthers from treated buds were smeared with 1% propionocarmine stain and observed under light microscope. Pollen sterility was studied by staining freshly collected pollen grains from 5 randomly taken mature flower buds between 8.30 AM and 9.30 AM in 1% acetocarmine solution and presented in percentage.

RESULTS

Inheritance and allelic relationship of gene (s) controlling yellow flower mutation (Yfm)

In three successive generations (M_2-M_5) of selfing, the mutant bred true for yellow flower (Fig. 1A). In the crosses between blue flowered varieties (Fig. 1B) and this mutant (M_5), the flower colours of F_1 plants were blue. In the F_2 and test cross (F_1 × mutant) progenies, plants were classified in two distinct groups, fitting a 3:1 and 1:1 ratios of blue to yellow flower, respectively (Table 1).

Allelic relationship of *Yfm* with other flower colour mutations were studied in three different intermutant crosses (Table 1). The F_1 plants obtained from crosses between *Yfm* and BPWFM and between *Yfm* and WFM exhibited yellow flower. In the F_2 and corresponding test crosses (F_1 × BPWFM and F_1 × WFM) the trait segregated, showing good fit to 3:1 and 1:1 ratios of yellow flower to blue-patched white flower in the first cross and yellow flower to white flower in case of second cross, respectively (Table 1). On the other hand, crosses between *Yfm* and PVFM yielded F_1 plants with yellowish-brown flower (Fig. 1A, C, D). In F_2, four types of flower colours, namely, yellowish-brown, pale-violet, yellow and white appeared, showing good fit ($\chi^2 = 0.78$, df = 3 at 5% level) to 9 yellowish-brown: 3 pale-violet: 3 yellow: 1 white (Table 1).

FIG. 1. Grass pea (*Lathyrus sativus* L.) plant; flowering twig of (A) yellow flower mutation, (B) blue-flowered control variety BioR-231, (C) pale-violet flower mutant and (D) an F_1 plant of pale-violet flower mutant × yellow flower mutant bearing characteristic yellowish-brown flower. Bars = 2 cm.

Segregation of Yfm in primary trisomics

Association of *Yfm* gene with a specific chromosome was elucidated by studying trisomic segregation of this recessive mutation. All the seven trisomics were self-fertile, and produced normal blue flower. Results in Table 2 indicated that the segregation of normal blue flower and recessive yellow flower in the diploid portion of F_2 progenies obtained from the cross between yellow flower mutant and trisomic VI showed significant deviation from the expected normal Mendelian disomic ratio of 3:1, but fitted well to the expected trisomic ratio of 8:1. The segregation ratio in BC_1 generation also exhibited deviation from the expected disomic ratio of 1:1, instead showed good fit to trisomic ratio of 2:1. The deviations from the normal disomic segregation ratios in both F_2 and BC_1 generations are ascribed to phenomenon of primary trisomy. No recessive homozygote plant was found in trisomic portion of F_2 population of this cross. On the other hand, segregation of dominant blue and recessive yellow flower in rest of the crosses between yellow flower mutant and trisomic I-V, VII showed good fit to 3:1, but exhibited strong deviation from the trisomic ratio of 8:1 in diploid portion of F_2 population (Table 2). Also, a good number of recessive homozygote plants in 2n + 1 portion of these six crosses were trisomic plants as confirmed by the presence of one extra chromosome (2n + 1; 2n = 15) in their genomes.

Segregation and chromosome association of translocation heterozygote in selfed progeny

Meiosis I chromosome association of the offspring obtained from the three consecutive selfed generations of RT-6 (*Yfm*) revealed that on an average the level of translocation heterozygosity in population has been maintained at about 47%, and the variation of this level between generations was not significant (data not shown). Out of total 580 plants obtained from selfed progeny of RT- 6 in three successive generations (M_2-M_5), 274 plants (47.24%) were cytologically confirmed as translocation heterozygotes showing 1IV+5II association at metaphase I and semisterile pollen, whereas 299 plants (51.55%) manifesting uniform presence of 7 bivalents and normal pollen fertility were categorized as homozygous fertile plants (N/N + T/T). Segregation of fertile and semisterile plants exhibited good fit ($\chi^2 = 1.09$, df = 1, at 5% level) to 1:1 ratio. Rest of the plants (1.21%) was confirmed as trisomics.

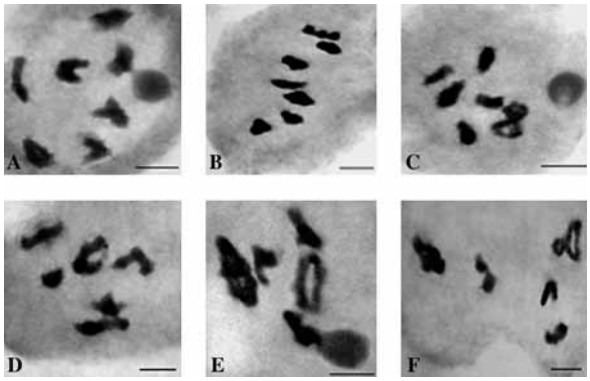

FIG. 2. Chromosome associations of normal (N/N), heterozygote RT-6 (N/T) and double heterozygote plants at diakinesis and metaphase I stages; 7II at diakinesis (A) and metaphase I (B) in normal nontranslocation (N/N) plants (2n = 14); one quadrivalent (8-shaped) and five bivalents (1IV + 5II) at diakinesis (C) in RT-6 (N/T); one quadrivalent (ring-shaped) and five bivalents (1IV + 5II) at metaphase I (D) in RT-6 (N/T); two quadrivalents (ring-shaped) and three bivalents (2 IV + 3II) at diakinesis (E) in double heterozygote plants; one ring-shaped and one 8-shaped quadrivalents with three bivalents (2 IV + 3 II) at metaphase I (F) in double heterozygote plants. Note that quadrivalent was not associated with nucleolus in double heterozygote plants (E). Bars = 10 μm.

As compared with regular occurrence of 7II (2n = 14) at meiosis I in four varieties and three induced mutant lines, the progeny RTs recovered from selfed RT-6 line exhibited quadrivalent association (1IV + 5II) at meiosis I (Fig. 2A-D). The RT line was true breeding for its characteristic yellow flower but constantly segregating in N/N, N/T, and T/T plants in self-pollination. In N/T plants, the percentage of pollen abortion ranged between 44.60% and 51.01% with mean value of 48.62%, and they showed quadrivalents at meiosis I. Among the quadrivalents, both the 8-shaped configuration characteristic of alternate or zig-zag type of orientation and open ring-shaped quadrivalent in adjacent type of segregation appeared in the progeny plants of RT-6 lines (Fig. 2 C, D). Out of 1200 total PMCs scored, 590 cells (49.17%) manifested 8-shaped configuration, whereas 552 PMCs (46.00%) exhibited ring-shaped structure at meiosis I. Rest of the cells possessed other types of associations, including trivalents, univalents etc. The trisomics were identified by the presence of a chain of 5 chromosomes, manifested in microsporocytes of seven plants (1.21%) at metaphase I. The weak growth habit of the trisomic plants was found associated with stunted stem, less number of branches and leaves, early maturity, higher pollen sterility (55.78-65%), and lower yield than normal diploid plants.

Detection of Translocation Homozygous (T/T) and Normal (N/N) Plants in Selfed RT-6

Careful observation of the 299 homozygous fertile plants led to the identification of 140 plants that showed weak growth habit as compared with rest of the 159 plants which was isolated as N/N type. Crosses between the plants manifesting weak growth habit and normal plants (N/N) resulted in F_1 progeny with increased pollen sterility (42.22-53.02%, mean 47.50 ± 2.3) over normal plants (1.40-1.59%, mean 1.53 ± 1.2). This identified the plants exhibiting weak phenotypes as T/T. These plants showed 7 bivalents (2n = 2x = 14) and low pollen sterility (2.0-2.95%) like normal plant, but could be distinguished from normal plants by retarded and slow growth accompanied with weak stem, small leaves and stipules, reduced number of leaves and branches and seed size. In contrast, F_1 plants of N/N × var. BioR-231were highly uniform in morphology, and showed 7II at metaphase I at regular occurrence. Thus, out of 580 plants recovered in selfed progeny of RT-6, 159 plants (27.41%) were N/N type, 274 plants (47.24%) were N/T type and 140 (24.14%) were T/T type, showing good fit ($\chi^2 = 2.35$, df = 2 at 0.05 level) to 1N/N : 2N/T : 1T/T. Seven plants (1.21%) were detected as trisomics and no segregation of yellow flower colour was found in the progeny.

Intercrossed Progeny: RT-6 × Normal varieties

The 270 F_1 plants obtained from the crosses between four normal nontranslocation cultivars (blue flower, N/N) and RT-6 (yellow flower, N/T) produced blue flower with normal phenotype, but cytological analysis revealed uniform presence of 7II in 137 plants (50.74%), association of 4 chromosomes (1IV + 5II) in 123 plants (45.56%), and pentavalent formation in 10 plants (3.70%). Range of pollen sterility (42.77-50.69%, mean 46.36 ± 5.4%) increased in these 123 plants. Segregation of normal fertile and semisterile plants showed good fit ($\chi^2 = 0.76$, df = 1 at 5% level) to the ratio of 1:1 in the intercrossed progeny (Table 4).

Mapping Yfm with translocation breakpoint

Linkage analyses between *Yfm* and a translocation breakpoint was carried out by joint segregation of these two traits in crosses between four normal homozygous non-translocation lines and RT-6 line (Table 3). Joint segregation of *Yfm* -breakpoint in four varieties × RT-6 exhibited large and significant ($p < 0.05$) χ^2 values for expected ratios in F_2 as well as in test cross progenies (Table 3). Pooled cross over value calculated from test cross data revealed a map distance of 10.31 cM between *Yfm* and the breakpoint (Table 3).

Test of independence in progeny of RT-6 × RT-(1-5)

In order to determine whether the translocated chromosomes involved in the RT-6 line were the same or different with the other five RT lines in grass pea, progeny from RT × RT was analyzed on the basis of quadrivalent configuration at meiosis I. Results for the five intercrosses were presented in Table 4. A close examination on metaphase I chromosome association of F_1 plants revealed occurrence of two rings of four chromosomes (2IV + 3II) in all the five crosses, and none of the rings was found associated with nucleolus during diakinesis (Fig. 2E, F). Among the total 610 F_1 plants of RT-6 × RT (1-5), exclusive presence of 7II was scored in 337 plants (55.25%), and it was followed by 171 (28.03%) single heterozygote and 102 (16.72%) double heterozygote plants. The single heterozygote population showing 1IV + 5II was actually the combination of 2 different RT heterozygote parents involved in cross. Association of two rings of four chromosomes with three bivalents was scored in microsporocytes of all the 102 double heterozygote plants (Table 4). Pollen sterility (69.11%) increased considerably in these double heterozygotes.

DISCUSSION

The yellow flower mutation (*Yfm*) was isolated in 200 Gy gamma ray irradiated M_2 progeny of grass pea variety BioR-231. This mutant is novel for two reasons: firstly, yellow colour of petal is a rare event in *Lathyrus sativus* L., and secondly, the mutant is carrying a chromosomal translocation. Inheritance pattern has been ascertained by crossing this mutant reciprocally with four grass pea varieties showing blue flower. Test of allelism was conducted between *Yfm* and three different flower colour mutant lines, exhibiting pale-violet, blue-patched white and white flower. The presence of blue flower in F_1 plants and a good fit of blue: yellow to 3:1 in F_2 and to 1:1 in test cross suggested that *Yfm* originated as a result of recessive mutation, and it was controlled by single locus. Two different non-allelic loci, B^+ and *Pv*, have been identified by earlier studies, where blue (B^+) was completely dominant over blue-patched white (*Bpw* allele) and white (*w* allele) colour of petals in grass pea (Talukdar &

Biswas, 2007a). Allelism test of *Yfm* with three flower colour mutant lines suggested that *Yfm* was completely dominant over blue-patched white flower and also, on white flower. Therefore, *Yfm* is an allele of B^+ locus which was multiple allelic, showing order of dominance $B^+ > yfl > Bpw$ and *w*. Interaction between *Yfm* and *Pv* locus, however, was non-allelic. This was evidenced by the appearance of a completely new flower colour phenotype, yellowish-brown, in F_1 and its segregation to yellowish-brown, pale-violet, yellow and white types of flowers in F_2. A good fit of this segregation to 9:3:3:1 supported the independent nature of *Pv* locus with B^+ locus in controlling the flower colour phenotypes in grass pea.

The value of a mutant trait is enhanced with knowledge of its location in the genomes (Kohel *et al.*, 2002). One of the important tools in chromosomal location of a particular mutation is the use of primary trisomic segregation of recessive phenotypes in F_2 and BC_1 generations (Hermsen, 1970). Isolation and subsequent characterization of seven different primary trisomics in grass pea have greatly enhanced the opportunity of linkage mapping of various traits in this crop (Talukdar, 2009a, c). All the seven trisomics are self-fertile. In the present material, chromosomal location of *Yfm* mutation has been elucidated by crossing trisomics as female parent and yellow flower mutant as male parent. A good fit of dominant blue and recessive yellow flower phenotype to trisomic ratio of 8:1 in F_2 and 2:1 in BC_1 in diploid portion of population obtained from crosses between *Yfm* × trisomic VI indicated that *Yfm* was located on extra chromosome of trisomic VI. So far, no gene has been mapped on this chromosome in grass pea. Thus, a new linkage group has been introduced in the present study.

Two different types of inheritance patterns were studied in the present mutant line. The *Yfm* was inherited as a true breeding recessive mutation, and no segregation within type was found in selfed progeny. However, a thorough meiotic analysis pointed out presence of three cytologically different types of plants in selfed progeny: normal nontranslocation (N/N), translocation heterozygote (N/T) and translocation homozygote (T/T). Range of pollen sterility (44.60-51.01%) indicated that the present RT-6 was semisterile in nature. Occurrence of 1IV+5II chromosome association at meiosis I confirmed that these semisterile plants were heterozygous for reciprocal translocation. Alternate or zig-zag orientation produced balanced and fertile gametes through 8-shaped chromosome configuration, whereas adjacent segregation showing ring-shaped quadrivalent at meiosis I resulted in formation of non-viable gametes due to duplication and deficiency. When these 2 types of segregations are of equal frequency, semisterility resulted (Endrizzi, 1974). Nearly equal frequency of alternate orientation (49.17%) and adjacent orientation (46.00%) might be responsible for 48.62% pollen sterility and 47% transmission rate in the present RT-6 line. In a population of four RT lines in grass pea, transmission rate between 42.00-52.56% in N/T plants has been reported, and a relationship between frequency of alternate orientation, pollen sterility and transmission rate has been drawn (Talukdar, 2010a). Clearly, alternate type of segregation compensated the loss of fertility due to formation of duplication and deficiency type of gametes in adjacent type segregation in the present RT-6 line, and justified the above contention.

In the present material, detection of 24.31% T/T plants in selfed progeny of RT-6 line was possible only when they were crossed with N/N plants. The resultant F_1 plants were semisterile (pollen sterility 47.50%). Predominant occurrence of 1IV+5II association at diakinesis and metaphase I of these F_1 plants suggested that the parental plants showing weak growth habit was carrying translocation in homozygous condition. These plants exhibited seven bivalents at meiosis I, but produced gametes with the translocated chromosome. As usual, N/N plants produced gametes with standard nontranslocated chromosomes. However, union of 2 different types of gametes (nontranslocated from N/N and translocated from T/T plants) led to disturbances in normal meiotic pairing resulting in quadrivalent formation and subsequent semisterility in F_1 plants. These F_1 plants (2n = 14) were indeed translocation heterozygote types, and one of their parents showing weak growth habit was actually translocation homozygote. In comparison to N/N and N/T plants, these T/T plants were weaker in growth habit and accompanied with low seed yield, but they were self-fertile and served as an important source of N/T plants in crosses with N/N plants. The homozygote inferiority exhibited in the present T/T plants was also found in pea (Müller, 1975), but it was in contrast with the reports of vigorous translocation homozygote in different other crops including soybean (Gale & Rees, 1971; Sjödin, 1971; Palmer & Heer, 1984).

Appearance of N/T plants was also evident in intercrossed progeny of fertile N/N (four varieties) and semisterile N/T plants. Results in Table 4 indicated

45.56% transmission of translocation heterozygosity in intercrossed population. This rate was very close to the transmission rate of RT heterozygote in selfed progeny. Presumably, the gametes carrying the translocated chromosomes functioned efficiently to some extent resulting in formation of viable seeds in progeny N/T plants. Like selfed progeny, the counterbalancing effect of alternate chromosome orientation over adjacent type might play a role in restoring partial fertility in these N/T plants in intercrossed progeny.

Significantly, a very low frequency of aneuploid plants appeared both in the selfed (1.21%) and intercrossed (3.70%) progeny. Regular presence of one pentavalent in microsporocytes of these aneuploids indicated origin of trisomy with translocated chromosome as extra in the present material. This result was in agreement with occurrence of trisomy in selfed as well as intercrossed progeny of four RTs described earlier in grass pea (Talukdar, 2010a), and was also reported in other legumes (Sutton, 1939; Pellew, 1940; Müller, 1975; Mercykutty & Kumar, 1985). Unequal 3:1 separation in the quadrivalent ring of RT-6 line at anaphase I might be responsible for formation of aneuploid gametes, leading to origin of trisomic condition in intercrossed progeny. The meiotic disturbances due to presence of one extra interchanged chromosome brought about higher pollen sterility, weak growth habit and very poor seed yield in these trisomic plants. Low frequency of trisomic plants, however, suggested predominant occurrence of 2:2 segregation over 3:1 separation in the quadrivalent ring of present material.

A map distance of 10.31 cM has been estimated between *Yfm* locus and translocation breakpoint in the present RT-6 line. Four varieties were used in crossing with RT-6 line to get more accurate result. Test cross data from these four crosses (being homogeneous) were pooled and map distance was presented. The result suggested linkage association between the two loci, and confirmed the presence of breakpoint on the same linkage group with *Yfm*. Among other leguminous crops, linkage association of translocation breakpoint was detected with a male-sterility gene in soybean (Sacks & Sadanaga, 1984), with phenotype of twisted tendril in pea (Gorel *et al*., 1992) and different isozyme markers in lens (Tadmor *et al*., 1987).

Occurrence of two rings of four chromosomes (2IV+3II) at metaphase I in the crosses between RT-6 and five previously isolated reciprocal translocation lines strongly suggested involvement of two different non-homologous chromosomes in exchange of their parts in the present RT-6 line. Formation of 2 rings of 4 chromosomes at metaphase I suggested absence of common chromosomes between the translocation lines involved in the crosses (Burnham *et al*., 1954). Exclusive presence of one ring of 4 chromosomes with five (1IV+5II) bivalents, however, was not found in progeny of any of the five crosses in the present material. The result indicated independent nature of the present RT-6 line from rest of the five lines, RT-1, RT-2, RT-3, RT-4 and RT-5, previously characterized in grass pea (Talukdar, 2010a). Five chromosomes (n = 7) were found to be involved in these five RT lines. Certainly, chromosomes involved in translocation of RT-6 line were completely different. Moreover, absence of association between nucleolar organizer region (NOR) and quadrivalent rings suggested that NOR chromosome was not involved in any way in translocation of RT-6 line. Preliminary cytogenetic analysis including conventional orcein-banded karyotype of root tip divisions and DNA base-specific CMA/DAPI banding in nontranslocation as well as RT lines pointed out involvement of chromosomes 1 (the NOR chromosome), 2, 3, 4 and 7 in translocation of five RT (1-5) lines in different combinations (Talukdar 2010a, b). The present RT-6 line is carrying a completely different translocation from rest of the five RT lines as evidenced by the presence of two rings in hybrid plants. Therefore, on the basis of this observation and preliminary cytological data, it can be assumed that the rest two chromosomes, 5 and 6, are involved in translocation of the present RT-6 line. Further study, however, is absolutely needed to verify the identity of the translocated chromosomes in RT-6.

In the present investigation, genetic basis of *Yfm*, its allelic relationship with other flower colour mutants, detection and cytogenetic characterization of a completely new type of translocation, its pattern of transmission and nature of independence have been elucidated. The study also revealed a linkage association between the locus *Yfm* controlling yellow flower and the translocation on extra chromosome of trisomic VI. Isolation of trisomic plants with interchange chromosome as extra is an important achievement of this study. The present RT-6 line with yellow flower as a phenotypic marker is a novel addition to chromosomal structural polymorphism and along with aneuploids may be used as excellent cytogenetic tools in future classical and molecular linkage studies of grass pea.

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