New mutations in the linkage group V have provided stable trisomic lines of pea

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As early as 1939 it was shown that selfing of a heterozygote for the Hammarlund translocation produced trisomics at a relatively high frequency (up to 18%) (4). The trisomic karyotype could be represented by seven pairs of chromosomes of the standard complement plus a small interchange chromosome composed of the short arms of chromosomes "II" and "V." (In this paper we need to refer to chromosomes and their markers; thus we will use the linkage group numbers for designation of the corresponding chromosomes). Although viability of these trisomics is lower than that of their diploid counterparts, they are quite fertile and are easily bred. In 1992 we made the cross WL1238 x SGE182 (1). From the F_2 generation a plant with a *cri*, *gp* phenotype was selected and crossed with the line WL1476 bearing the Hammarlund trans-location. After an insuing test-cross (No. 3 in ref. 1), with a *cri*, *gp* plant as a tester, we obtained a trisomic TRIS-54 in which both chromo-somes V carried the recessive allele *cri*, while the extra chromosome possessed the wild type allele *Cri* in the vicinity of the breakpoint (BP) (Fig. 1).

Among the progeny generated from TRIS-54 we could easily distinguish diploid plants (genotype *cri*/*cri*) from trisomics (genotype *cri*/*cri*/*Cri*) even as seedlings (the symbols of genes residing on the extra chromosome are given last. Additional evidence for the presence of the extra chromosome included enlarged bracts, larger average number of flowers on inflorescences, and undulating margins of stipulae and leaflets. In problematic cases a microscopic analysis of bivalents in PMC was performed. The proportion of tisomics in progenies of trisomics varied between 10-20%, so that selfing permitted a considerable increase in the number of trisomic lines. In an overwhelming majority of cases, diploid plants resulting from selfing of trisomics had the phenotype *cri*, but among 1052

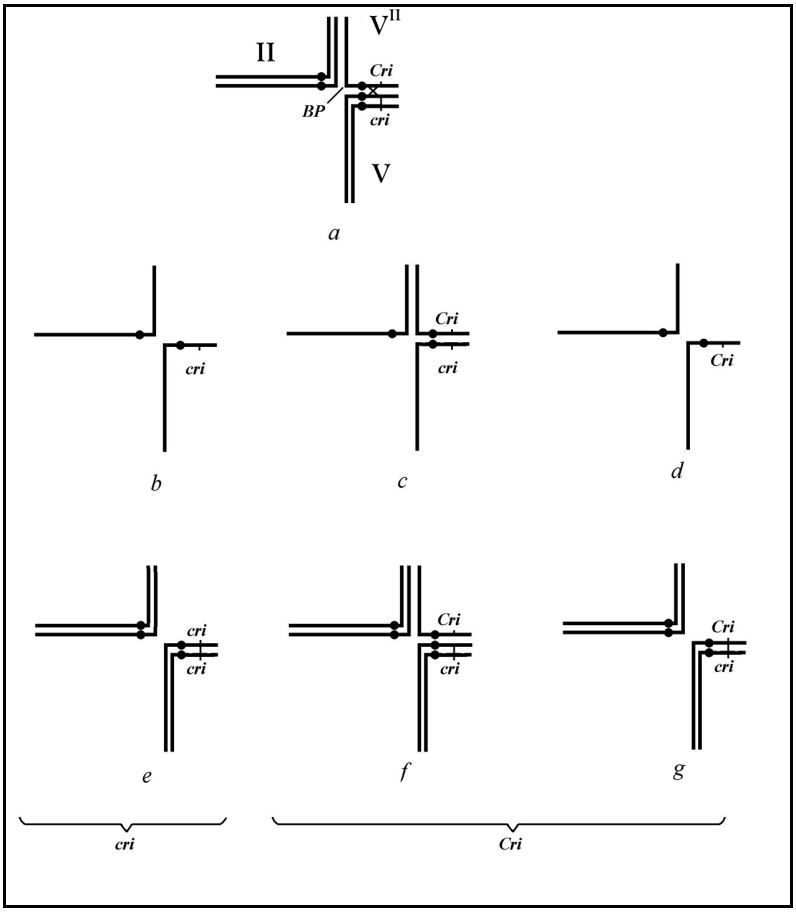

Fig. 1. Scheme of inheritance of the extra chromosome in the line of tertiary trisomic TRUST. Numbers stand for: II—chromosome II; V—chromosome V; V^{II}—small interchange chromosome, composed of the short arms of chromosomes II and V. a—original tertiary trisomic; b—euploid gametophyte; c—hyperploid gametophyte; d—euploid gametophyte carrying a recombinant chromosome. Crossing-over occurred between translocation breakpoint (BP) and the cri *marker. e—normal diploid* cri/cri*; f—trisomic* cri/cri/Cri*, g—recombinant diploid*

diploids obtained from trisomic descendants of TRIS-54 we recorded four recombinant plants with the phenotype *Cri*. Thus, the fraction of normal chromosomes V that recombined with the additional interchange chromosome between the locus Cri and BP was 0.19%. The recombinant plants differed from trisomics by a greater vigor and fertility, the bracts were missing and stipulae and leaflets lacked the characteristic waviness. The proportion of *cri* plants in the progeny of the recombinant plants was approximately 25%.

To introduce additional markers into the short arm of chromosome V we crossed a trisomic plant (with phenotype *u*), resulting from two generations of selfing of TRIS-54, with a diploid plant with the genotype *cri Ust*/*cri Ust*, originating from the above mentioned cross WL1238 x SGE182 (1). From the resulting F_2 a trisomic with the phenotype *Ust* was selected. This plant was the founder of the near-isogenic trisomic line 'TRUST.' This line was increased for several generations, with selection of trisomics in each until several tens of thousands of plants had been produced. The proportion of trisomics in each generations of this line was relatively high (20-30%).

The most striking peculiarity of the line TRUST was an absence of diploids with the phenotype Cri, indicating suppression of recombination between *Cri* and BP of the Hammarlund translocation. A present we have scored more than 10,000 individulas and have not found a single recombinant. The phenotype of TRUST was closer to normal than the trisomic descendants of TRIS-54: the leaflet margins were less wavy and pollen fertility and number of seeds per pod were greater. TRUST trisomics have less than 10% sterile pollen grains as compared to 10-20% in TRIS-54. Nevertheless, in addition to seven bivalents a small univalent chromosome was invariably observed in PMC. In reciprocal crosses of the line TRUST with the testerline YELLOW CRISPA we determined that the extra chromosome was transferred via the female gametophyte at a frequency of 25.5% (sample size N = 383) and via the male gametophyte with a frequenacy of 12.3% (N = 983).

We attempted to introduce into the genome of the line TRUST additional recessive mutations that would be manifested only in diploid plants with the phenotype *cri* and covered by normal alleles in trisomics, with the phenotype *Cri*. Approximately 2800 seeds produced by TRUST trisomics were left to imbibe in 0.1% EMS solution. At the seedling stage plants with the phenotype *cri* were removed and the remaining 789 plants grown in a greenhouse. For the majority of plants, seed productivity appeared to be very low. The seeds collected from the 210 most productive M1 plants were sowed in the greenhouse. From each family a single M_2 trisomic plant exhibiting no visible mutations was chosen, and the progenies of such plants were planted in the field. By selecting the plants with the normal phenotype we tried to eliminate, at least in part, induced mutations not covered by the extra chromosome. Among 207 M3 families 43 displayed various mutations but only three of these exhibited a strong linkage with cri. One, called *fas*3, had a fasciated stem, while two other, called *killer* (*kil*) and *remover* (*rmv*) were believed to be recessive lethals because no plants of the phenotype *cri* were observed in the families where they were found. The trisomic carries of the mutations *rmv* and kil became founders of the lines TRUST-R and TRUST-K, respectively.

The line TRUST-K does not differe from the initial line TRUST, either by the phenotype of trisomics, by pollen fertility, or by number of seeds per pod. However, after imbibition only about 25% of the seeds germinate to give rise to trisomics, while the germination process of the remaining 75% of the seeds stops when the radicals are about 5 mm long. A screening of 1012 seedlings of the line TRUST-K with the genotype *cri kil*/*cri kil*/*Cri Kil* revealed only one diploid plant with the phenotype *cri*. Thus, the fraction of the normal chromosome V recombining with the extra chromosome between *Kil* and *Cri* was 0.05%.

Two plants of the line TRUST-K were crossed with the line WHAF (crd^{wh}, *a*, *af*) described in ref. 2. Two resulting F_1 plants wre diploids with the phenotype (*A*, *Cri*). Of 154 F_2 plants examined, only six had the phenotype cri, indicating that the location of the sporophytic lethal *kil* is on the short arm of chromosome V about 6.0 $\pm$ 1.4 cM from the locus *Cri* (computed by the maximum likelihood method using equations from ref. 3). Thus, in trisomics the recombination distance between the loci *Cri* and *Kil* is reduced at least 100-fold.

The line TRUST-R is remarkable in that all its seeds germinate and give rise only to trisomics *cri rmv*/*cri rmv*/ *Cri Rmv*. The number of seeds per pod in this lines is reduced by half. Most probably, the diploids die at an early stage of embryo development. Screening of more than a thousand plants of this line failed to identify a single plant with the phenotype *cri*, giving a recombination distance between *Cri* and *Rmv* of less than 0.05 cM.

A plant of the line TRUST-R was crossed with the line WHAF. An F_1 plant was trisomic with the genotype *cri rmv*/*Cri Rmv*/*Cri Rmv*. In this plant recombination between the chromosomes of the basic complement could easily occur, but among 60 F_2 plants none with the phenotype *cri* was found. It is evident that the lethal *rmv* is tightly linked to *cri*. In addition, we obtained three other lines for which the yield of trisomics had changed significantly. In two of them, TRUST-6 and TRUST-138, the proportion of trisomics was about 50%, wihle in the line TRUST-111 the fraction was 90%. At present we are investigating the nature of the mutations underlying this increase in the proportion of trisomics.

In summary, based on the Hammarlund translocation we have created a genetic system allowing easily differentiation between diploids and trisomics for a short interchange chromosome with little effect on viability and fertility. Due to an unknown event that occurred between TRIS-54 and TRUST, the ability of this extra chromosome to recombine with those of the normal karyotype has been sharply reduced. Finally, through the use of chemical mutagenesis we induced two lethals that exclude diploids from the progeny, in effect producing two stable trisomic lines.

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