Identification of a DNA marker closely linked to the nitrogen fixation mutant *sym26* and its assignment to an unclassified linkage group.

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The non-fixing mutant line P63 was initially isolated and described by Duc et al. (1) along with several other mutants that produced the same white-nodule phenotype when exposed to *Rhizobium leguminosarum* strain 128C53. Complementation analysis suggests that this mutant is not allelic with other non-fixing mutations [(1) and G. Duc, pers. comm.] and has been assigned the gene symbol *sym26.* We have been attempting to map each of these mutants by crossing with the line JI73 (= NGB1238), performing bulked segregant analysis on segregating F_2 or F_3 populations derived from these crosses, and mapping the markers identified in a standard recombinant inbred population derived from the cross Sparkle x JI73. Although Due's mutants were produced in cv. Frisson, the same DNA markers generally segregate in both crosses.

The initial P63 x JI73 F_2 population consisted of 53 plants which were scored for nodulation phenotype as described previously (2). The mutant phenotype was clearly expressed, with 12 of the 53 plants displaying the mutant phenotype. Progeny from 14 of the F_2 plants with normal phenotype were further examined for incidence of the mutation. Ten of these progenies segregated for the mutation. The F3 results confirmed the recessive nature of the mutation and identified four progenies which were apparently homozygous wild type. DNA was extracted from a single individual from each of these four F_3 populations and from five F_3 plants derived from five different non-fixing F_2 plants. These DNA samples were combined into two bulks (wild type and non-fixing) and used for bulked segregant analysis (BSA) (3).

Approximately 100 10-base oligonucleotides were tested using BSA. The primers were selected to cover all linkage groups developed in the Sparkle x JI73 recombinant inbred lines described in (4). One 425-bp fragment produced by primer OPA09 was present in JI73 and the wild type bulk but not in P63 or the mutant bulk. Further analysis of 21 F_4 plants derived from 19 different F_2 plants by single seed descent gave no recombinants between the marker and the gene. The OPA09425 marker belongs to a linkage group (linkage group 11) that has yet to be matched with any of classical linkage groups in pea. However, it is likely to be part of linkage group IVA or VI because most of the remaining known linkage map has already been paired with other linkage groups identified in this population. None of the markers on the remaining linkage groups gave rise to clear differences between the fix- and wild type bulks. The position of the mutant was further confirmed by testing markers adjacent to OPA09425 on linkage group 11, as developed for the Sparkle x JI73 RILs (Fig. 1). One of these markers, BC213a, segregated in the P63 x JI73 F_3 population and displayed close linkage with the wild type phenotype.

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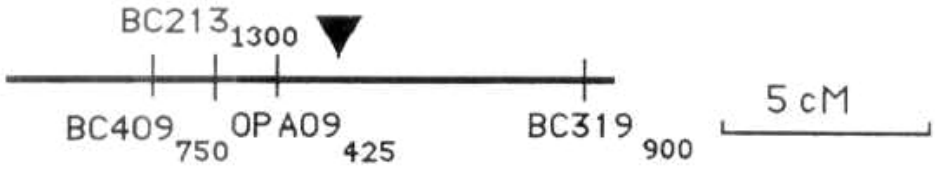

Fig. 1. Linkage group 11 of the Sparkle x JI73 RIL map. The approximate position of *sym26* is identified by the triangle. The scale is given on the right.

Acknowledgements. This research was funded in part by USDA-NRI and The International Atomic Energy Agency.

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